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PHYSIOLOGICAL ANALYSIS OF THE GENERAL CORTEX IN REPTILES AND BIRDS*

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(Received for publication February 18, 1939)

I. INTRODUCTION

THE FUNCTIONAL significance of the so-called general cortex of reptiles and birds is not well understood. Physiological studies are few and the use of the oscillograph for study of the cortex of these submammalian forms has not been attempted. The extensive and frequently controversial literature concerning this part of the telencephalon will not be reviewed; a brief description of the principal anatomical facts is, however, essential for orientation. For a complete and critical treatment of the anatomical problems the reader is referred to the work of Kappers, Huber, and Crosby (1936), and for physiological literature to the recent reviews of ten Cate (1936, 1937). Previous work directly concerned with the present experiments of stimulation and oscillographic recording is discussed.

ANATOMY

Reptiles. The general cortex of reptiles, or that part of the surface area not primarily olfactory in character, lies in the dorsal lateral surface of the cerebral hemisphere. Its medial boundary is formed by the hippocampal region and its lateral boundary by the piriform lobe. It is in intimate relation by means of a primordial neopallium with the hypopallial region which is probably homologous to certain parts of the corpus striatum of mammals. Dart (1935) has subdivided this general cortex into a medial para-hippocampal portion and a lateral para-piriform area. One of the most complete studies of the anatomy of the reptile telencephalon is that of Johnston (1915) on the turtle. This rather primitive reptile is more easily homologized with the amphibian forms and primitive mammals than are certain other species which seem to have developed along avian lines. Johnston was able to outline a general cortex extending over the dorsal and lateral surfaces of the hemisphere. This area separates the hippocampal and piriform areas except at the rostral pole where these olfactory regions fuse. He felt that the medial part of the cortex was principally sensory, while the lateral and anterior parts were mainly concerned with motor function, but recognized the probability of considerable overlapping between the two.

Besides the interconnections between the general cortex and its contiguous areas, both cortical and hypopallian, this area has numerous afferent and efferent connections with the diencephalon and mesencephalon. Various workers have described four tracts from the hippocampus and associated regions going by way of the median forebrain bundle to the hypothalamus and adjacent areas of the mesencephalon. These are considered predominantly efferent in respect to the cortical areas and include within them connections homologous to the fornis and fornix lungus systems of mammals and the septo-mesencephalic system in the bird. There are numerous pallial commissures connecting the olfactory regions of the two hemispheres. The question of the presence or absence of a true corpus

* The term general cortex or general pallium was introduced by Johnston in 1915 in his description of the turtle brain. Kappers, Huber and Crosby (p. 1338) state "The term general cortex . . . is applied to those cortical areas which are concerned primarily with impulses other than olfactory, although such general cortex will be associated by fiber bundles with the developing olfactory cortices medial and lateral to it."

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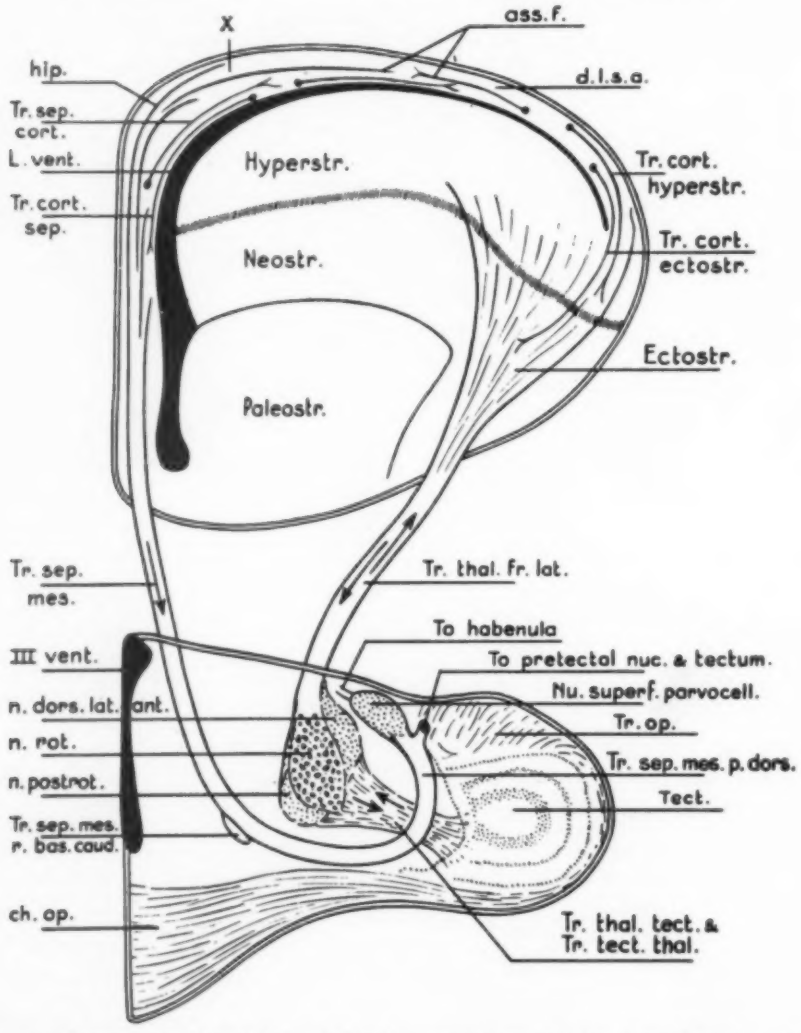

FIG. 1. Diagram to show the principal connections of the dorsolateral surface area (corticoid area) in the pigeon. (Modified from Kappers, Huber and Crosby, 1936.) *ass. f.*, association fibers; *ch. op.*, chiasma opticum; *d.l.s.a.*, dorsolateral surface area; *ectostr.*, ectostriatum; *hip.*, hippocampus; *hyperstr.*, hyperstriatum; *l. vent.*, lateral ventricle; *n. dors. lat. ant.*, nucleus dorsolateralis anterior; *n. postrot.*, nucleus postrotundus; *n. rot.*, nucleus rotundus; *n. superf. parvocell.*, nucleus superficialis parvocellularis (nucleus of the tractus septo-mesencephalicus); *neostr.*, neostriatum: *paleostr.*, paleostriatum; *tect.*, tectum; *tr. cort. sep.*, tractus corticoseptalis; *tr. cort. hyperstr.*, dorsolateral surface area component to the hyperstriatum; *tr. cort. ectostr.*, dorsolateral surface area component to the ectostriatum; *tr. op.*, tractus opticus; *tr. sep. cort.*, tractus septocorticalis; *tr. sep. mes.*, tractus (cortico-) septo-mesencephalicus; *tr. sep. mes. p. dors.*, tractus (cortico-) septomesencephalicus pars dorsalis; *tr. sep. mes. r. bas. caud.*, tractus (cortico-) septo-mesencephalicus ramus basalis caudalis; *tr. thal. fr. lat.*, tractus thalamo-frontalis lateralis; *tr. thal. tect.* and *tr. tect. thal.*, tractus thalamo-tectalis and tractus tecto-thalamicus. *III vent.*, third ventricle; *X*, area of uncertain homology. (See Kappers, Huber and Crosby, 1936.) The connections between the dorsolateral surface area and the hyperstriatum accessorium are not shown. The olfactory connections of the septal region are not shown, nor is the tractus septo-mesencephalicus ramus basalis frontalis indicated.

callosum in these forms is unsettled and must await the application of degeneration experiments. Numerous connections between the cortex and the habenular region have been described within the stria medullaris. The principal non-olfactory afferent connection to the general cortex is probably carried by ascending tracts in the lateral forebrain bundle, which is well developed in reptiles. Of particular importance are the fibers in the extreme lateral part of this complex bundle. Shanklin (1930) has described a direct connection between the dorsolateral cortex and the nucleus rotundus, but this has not yet been found by other workers. The paths carrying the impulses responsible for the motor movements produced by stimulation of the dorsal pallium in reptiles have not been demonstrated (Kappers, Huber, and Crosby, 1936). Association fibers within the general cortex area are well developed. The question of cortical lamination in reptiles is still one of controversy. Its identification apparently depends both on the criteria considered essential for identifying such structural organization by different investigators and on the species examined. Lamination in the mammalian sense is not present in the general cortex of the turtle.

Birds. The telencephalon of birds differs from that of the reptiles in the relative reduction of the olfactory connections in most species, and in a great development and differentiation of the corpus striatum. This has gone hand-in-hand with the increased differentiation of the dorsal thalamus and other somatic centers, particularly the optic system. The general cortex has not kept pace with the striatal development, and as a result shows marked differences in various species. In most, however, the general cortex of reptiles is represented in birds by a thin band of corticoid tissue, the so-called dorsolateral surface area (Fig. 1). Edinger, Wallenberg, and Holmes (1903) and others have labelled in addition the hyperstriatum accessorium and the dorsal hyperstriatum, as cortex. Following the definitions of the neopallium as given by the students of mammalian cytoarchitectonics, Rose (1914) maintained that there is no avian neopallium. It is obvious that an opinion whether there is or is not a neopallium depends on one's definition of the term. The importance of comparative physiological studies to supplement anatomical observations is clear. The brain of the pigeon has been exhaustively studied by Edinger, Wallenberg, and Holmes (1903) in an admirable comparative anatomical work and by Huber and Crosby (1929).

The connections of the avian telencephalon have been determined by degeneration experiments, as well as by the silver and myelin staining methods. The principal connections of the dorsolateral surface area are shown in Fig. 1. The first tract to be described was the cortico-septomesencephalic tract. It has been studied by degeneration experiments (Boyce and Warrington, 1898; Edinger and Wallenberg, 1899; and Wallenberg, 1906, and others); and the facts of its origin and termination are well established. It is an efferent tract in respect to the "cortex" and distributes to the hypothalamic regions and to the basal regions of the mesencephalon and even the medulla in certain species by its basal ramus. The tectum and the habenula receive its dorsal ramus. Though not morphologically resembling the pyramidal system of mammals, it may well serve a similar function in the avian forms. The other connection with the lower centers is the lateral thalamofrontal tract. This is probably both ascending and descending, although the tract has not been studied as systematically as the septo-mesencephalic. The lateral thalamofrontal tract probably provides a connection to and from the well developed tectal region and the "cortex" by way of the nucleus rotundus and other neighboring nuclei. Its lateral location in respect to the ventricle and its striatal course resembles the internal capsule system of mammals. Its functional significance, although probably important, is not well known, but it is thought that the ascending connections are of chief importance. Short association neurons are found within the dorsolateral surface area and connections to the adjacent areas of hippocampal cortex and striatum are numerous and undoubtedly important functionally (Fig. 1).

It is obvious that although the avian telencephalon bears a certain resemblance to the mammalian, its homologies are less clear than are those of the more primitive reptilian telencephalon. The "cortex" has apparently become vestigial to some extent, while the striatum has developed in complexity and differentiation, and parts of it probably serve as a vicarious cortex. As we shall see, however, although anatomically less well developed, the corticoid layer of birds still bears a functional relationship to the homologous parts of the mammalian brains.

PHYSIOLOGY

Reptiles. Properly speaking, there is no physiological literature on the cerebral cortex of reptiles. The small size of the cortex and its intimate union with subcortical centers has rendered a selective study of the general cortex extremely difficult. This has forced us to consider the reported physiology of the entire forebrain and to attempt to separate that part which concerns the cortex proper. The experiments of *ablation* are inconclusive. The older studies of Fontana, Rolando, and Desmoulins were inconclusive (ten Cate, 1937). In the turtle, after ablation of the cerebral hemispheres, spontaneous movements were less frequent (Fano, 1884, and Bickel, 1901), but nevertheless possible (Sergi, 1904). Following such ablations in the adder, according to Schrader (1892), there were no longer movements corresponding to various emotional states such as fear, rage, etc. The responses to light stimulation were normal (Bickel, 1901).

Stimulation experiments have furnished more abundant results, the interpretation of which is difficult, because of the danger of spread of the current. Bickel (1901) never obtained any effect in the turtle with the use of weak electrical stimulation or with chemical stimulation. Only strong stimulation gave a response, and this was probably attributable to spread of current. Johnston (1916) on stimulation of the anterior part of the brain of the turtle obtained movements of the neck, eyes, jaw, extremities, and tail. Stimulation of the olfactory bulb and the striate body likewise induced these movements. The other parts of the forebrain were inexcitable. Analogous results were obtained in the lizard. The anterior part of the pallium has a particular histological structure and this might suggest that it was the excitable part of the forebrain. However, the fact that narcosis augments rather than suppresses the movements made him think that this action results from a spread of current. The observations of Koppányi and Pearcy (1925) confirm this point of view. These authors have not observed in the turtle movements from electrical stimulation, except when the electrodes were forced into the corpus striatum, and in this Tuge and Yazaki (1934) concur. They have confirmed in the turtle most of the phenomena described by Johnston (1916). Bagley and Richter (1924) and Bagley and Langworthy (1926) have described complex movements, never discrete, on stimulating a well localized part of the cerebral cortex of alligators. Narcosis diminishes the excitability of this zone, and, moreover, stimulation of both the striate body beneath and other cortical areas about the excitable area does not produce this effect. This difference in behavior between the alligator and the turtle may be explained by the fact that the brain of the latter is less well developed.

Birds. The same general remarks that have been made for reptiles apply here. Because of the relatively greater development of the striatum, ablation experiments, which have uniformly included these parts of the forebrain, have no value in the analysis of the function of the cortex in birds. The experiments of excitation are numerous, but opinion is divided concerning the excitability of the cortex. Ferrier (1876), confirmed by Stiner (1891) and Boyce and Warrington (1898), described an area in the superior parietal region of the brain, the stimulation of which provoked myosis of the contralateral eye and a rotation of the head to the opposite side. Gallerani and Lussana (1891) have also observed some movements of the head on chemical stimulation of the posterior part of the cerebral hemisphere. The most complete research is that of Kalischer (1900 a and b, 1901, and 1905), who studied carefully the electrical excitability of the cerebral cortex in various birds, particularly in the parrot. In this last species where the cerebral cortex is particularly well developed, Kalischer found evidence of true motor localization. Proceeding from a rostral point caudally, a focus was observed for tongue and jaw movements, a center for phonation, foci for movements of the foot and wings, and finally in the occipital region a zone was present which gave movements of the eyes.

Negative results are not lacking, however. Bickel (1898) has never been able to observe movements from weak stimulation, but only with the use of strong currents. He has considered them as attributable to spread of current to the striate body. Koppányi and Pearcy (1925) have likewise recently reported some negative results in the pigeon. Rogers (1922) working on the cerebral cortex of the pigeon, arrived at the same conclusion. He found only two movements of certain cortical origin, namely myosis of the contralateral eye and a depression of the feathers about the throat. All other movements were interpreted as being attributable to a spread of current to the corpus striatum. The divergence of results is striking, especially in so simple an experiment. The cause of the negative results is probably due to the use of the narcosis. Roger's experiments were performed under ether

anesthesia, and it is possible that this author has not obtained the responses of the most excitable centers, these having been depressed by the ether. The experiments of Bickel and those of Koppányi and Pearcy have all been done on unanesthetized animals. In these experiments, there may be possibly another cause of error, *i.e.*, the pain provoked by the immediately preceding operation.

MATERIAL AND METHODS

The pigeon and the turtle have been used as experimental animals. All observations of excitation and derivation of potentials have been made in the absence of all general anesthetics. The animals were prepared under ether narcosis generally some hours before the beginning of the experiment. An apparatus was constructed that could be affixed to the head of the animal and permitted the fixation of electrodes for stimulation or derivation but did not disturb the movements of the head. In experiments in which both stimulation and derivation were done, the same electrodes were used, but the preparation was shunted from the amplifiers. The animals were completely awake during the experiment, but were kept free from pain by the appropriate local use of 2 per cent cocaine.

The stimulation was either monopolar or bipolar. A faradic current with a frequency of about 40 to 50 per sec. was used. The derivation of potentials was always done with bipolar electrodes made of silver wires to which were attached fine cotton wicks soaked in Ringer's solution. The electrical activity was registered by means of an amplifier of five stages coupled with small capacities (0.1μF), and a Dubois oscillograph.

II. Experimental Studies

Stimulation

Turtle. Faradic stimulation of the brain provokes movements of the neck, jaw, and feet. The intensity of the stimulus is of the same order as that necessary to induce masticatory movements when applied to the masticatory area of the cerebral cortex of the normal rabbit. However, these motor effects in the turtle are certainly not cortical in origin. The use of cocaine applications indicates that they are probably due to a diffusion of current to subcortical centers. We were unable to abolish the responses in question by a very prolonged cocainization (2 per cent applied for 5 min.). It is known that 2 min. are sufficient to abolish the responses from the masticator cortex in the unanesthetized rabbit. It will be pointed out later that only 5 sec. of this cocainization are sufficient to abolish all motor responses from the cortex of pigeons.

Pigeon. The faradic stimulation of the cerebral cortex of the pigeon without general narcosis has given definite and conclusive results. Under these conditions, the excitability is high and the responses are readily abolished with the most superficial application of cocaine. The movements that were induced by weak stimulation of the dorsolateral surface area of the forebrain of the pigeon are the following: (i) Rotation of the head toward the side opposite to that stimulated. (ii) Conjugate movements of the eyes in the same direction. (Forward movement of the homolateral eye and a backward movement of the contralateral eye.) (iii) Myosis of the contralateral eye. (iv) Depression of the contralateral lower eyelid.

The first three movement were constantly present and usually simultaneous. The rotation of the head resembled a "spontaneous" movement of the animal. It might be carried to 180°. This movement of rotation often included a vertical component either upward or downward. The lowering

of the contralateral lower eyelid was not seen in every instance. Figure 2 shows the extent of the excitable area. We were unable to separate special centers within the excitable area for the different movements. The threshold of excitability was low. With bipolar stimulation (electrodes cotton wool wicks soaked in Ringer's solution) a distance of 25.5 cm. between the primary and the secondary coils of the inductorium usually gave a response. With the same apparatus, electrodes and frequency and strength of stimulation (2 V. in the primary and 50 shocks per sec.), the threshold of the masticator cortex in the waking rabbit is generally with the coils 24 cm. apart. The threshold is still lower, in the pigeon as in the rabbit, if a punctiform metallic monopolar electrode is used.

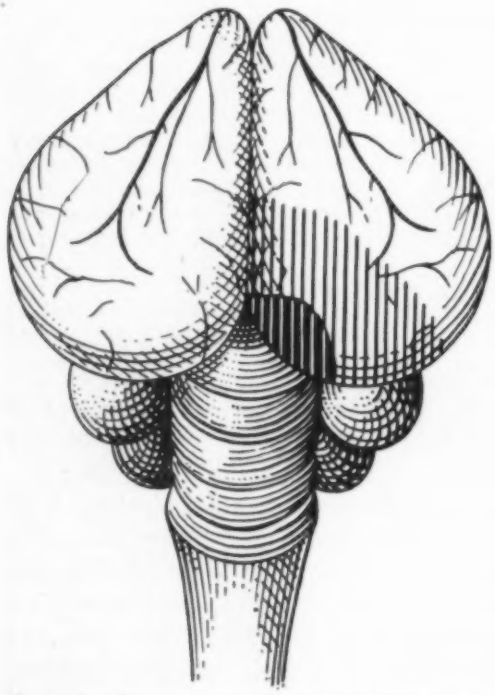

FIG. 2. The topography of the excitable zone of the cerebral hemispheres in the pigeon. The region with black stripes on a white background indicates the limits of the excitable area. That with white stripes on a black background is the region of maximal excitability.

If, having determined the intensity of the stimulation that was exactly supraliminar, the frequency of the breaks in the primary circuit were reduced from 50 to 2 per sec., a response was still seen. In these conditions however, instead of the response appearing in 2 sec. as in the first instance, it did not appear until the stimulation had been prolonged for 30 to 40 sec. With the stimulation at this low frequency, the response although it was long delayed in appearance, otherwise did not differ from the movement produced by the more frequent stimulation. The movement of rotation of the head did not show any discontinuity corresponding to each separate induction shock.

The excitability of the cerebral cortex is sensitive to outside agents. General narcosis of even light ether or dial reduces it considerably. In addition, in the absence of general narcosis, if pain was not prevented by the appropriate use of a local anesthetic (cocaine 2 per cent), it was observed that the responses were much less regular. This was perhaps caused by a nociceptor inhibitory reflex. We believe that these factors are the principal causes of the divergence between the reported observations by different workers on this problem.

All the above mentioned responses were immediately abolished by an extremely superficial cocainization. If a piece of filter paper only 5 mm. square, moistened in a 2 per cent solution of cocaine, was placed on an

excitable point for only 5 sec. the response from this point was completely suppressed. This procedure is much more rapid and exact than when cocaine is applied to the laminated cortex of mammals. The adjacent points of the cortex about the area cocainized remained excitable, showing that the effect of the cocaine was restricted to the site of its application. Another proof of the cortical nature of this response has been furnished by chance. One of the pigeons of this series had an atrophy of the cortical tissue on one side in the area giving the responses. (See protocol below.)

EXPERIMENT, PIGEON NO. 4

After a preliminary ether anesthesia, the left hemisphere was exposed in the usual manner by trephine and rongeur. On opening the dura it was obvious that the dorsolateral area of the occipital and parietal region was abnormal. The corticoid layer was thinner than normal, wrinkled and translucent. There was no sign of recent damage to the bone, meninges or skin over this area. The opposite hemisphere was exposed, as was customary, and was normal in gross appearance. Inasmuch as the atrophic area coincided so closely with the area from which movements of the head were obtained, we proceeded with the following experiment.

Technique. Inductorium with 2 V. in the primary and 50 break shocks per second. Monopolar metallic electrode at the point of maximal excitability. Duration of stimulation at each strength, 2 sec. As a test of response a movement of the head toward the contralateral side. For cocainization a piece of filter paper 5 mm. sq., moistened with 2 per cent cocaine. Responses were obtained from the normal side (right) with weak current while even prolonged stimulation with much stronger current on the atrophic (left) side failed to give any response. Cocainization abolished the response on the right side.

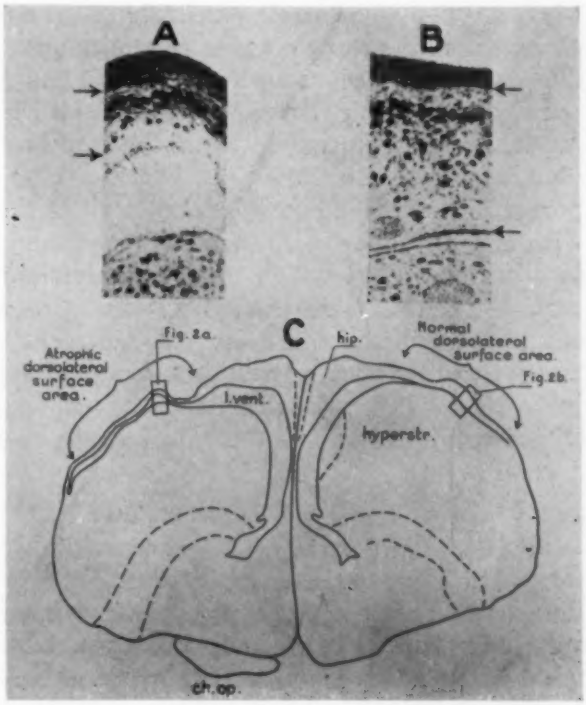

FIG. 3A. Section through the dorsolateral surface area on the left side (Expt. 4); the most superficial layer is a blood clot, the result of stimulation and trauma. The two arrows mark the limits of the corticoid layer. B. A section through the opposite side in the same area. Note the thickness of the corticoid layer as compared to 3A. C. Diagram of section from which photographs 2A and B were taken.

After the stimulation experiment, the superficial surface of both sides was hyperemic and slight lesions had unavoidably been made at one or two points on each side. The brain was carefully removed from the skull and placed in 95 per cent alcohol. After fixation it was cut transversely and examined grossly. The medial part of the tissue outside the ventricle was identical to its fellow of the opposite side; but the lateral portion, the dorsolateral surface area proper, was less than one-half the normal thickness. The frontal parts of the brain and the striatal areas, as well as the cerebellum and hindbrain, appeared to be normal and everywhere symmetrical. The brain was imbedded in celloidin and sectioned at

25 micra, every 12th section being mounted and stained by the Nissl technique for nerve cells.

Histological examination failed to reveal any abnormality of the frontal pole of the telencephalon, nor any of the striate areas, the extreme lateral or medial parts of the "cortex" or any lower center. However, from the most anterior point at which the dorsolateral surface area is separated from the underlying hyperstriatum by the lateral ventricle, the intermediate portion of the cortex, corresponding to the position of the general cortex in reptiles, was obviously thinner than its corresponding layer of the opposite side. Figure 3A and B, shows sections through corresponding portions of the atrophic and normal sides respectively, taken near the rostral border of the defect, at the junction of the anterior two-thirds and posterior third of the hemisphere. Figure 3c is a diagram of the entire section, showing the site of the microphotographs 3a and b. More posteriorly, the atrophic area is even thinner and in places indistinguishable from the underlying caudal neostriatum at low magnification.

The motor responses described are thus cortical in origin. To ascertain whether there are correlations between the two excitable areas on the two sides, an attempt was made to reproduce the phenomena of "secondary facilitation." In pigeons, however, the simultaneous stimulation with currents just subliminal was without effect. There is, therefore, probably no mutual reinforcement between the two cortices, and the absence of the phenomenon of secondary facilitation speaks against the existence of important intercortical correlations in these forms. Simultaneous stimulation with two physiologically equal but supraliminar stimuli resulted constantly in a curious effect which was probably the result of an interaction of the cortical excitation on the subcortical centers. In these circumstances, the animal turned its head neither to the right nor toward the left, but made movements of the head and neck toward the front, as if to peck at some object with its beak. All these movements, like those produced by unilateral stimulation, were similar in appearance to spontaneous movements of a normal animal.

Oscillographic studies

Turtle. The normal oscillogram in the unanesthetized turtle should be compared to a similar method of recording of the potentials derived from the exposed brain of an unanesthetized mammal. The rabbit has been the only animal previously studied (Ectors, 1936). The amplitude of the electrical potentials is much less in the turtle than in the rabbit. In general, we have been obliged to work with a sensitivity of 5 to 10 times that ordinarily employed in mammals. The aspect of the oscillogram is much more regular. One does not see, in contradistinction to the rabbit, the large waves comparable to the human electroencephalogram. In the turtle there is simply a succession of fairly regular electrical oscillations of a frequency analogous to the beta waves (about 40 to 50 per sec.) and of feeble amplitude (10 to 20μV., Fig. 4A).

The electrical activity thus recorded is not exclusively cortical. A small part of it does have its origin in the cells of the cortex proper, but the potentials of subcortical centers are recorded simultaneously. A superficial cocainization does not suppress or even reduce the activity considerably. After the application of 2 per cent cocaine for 5 to 15 min. there is still considerable spontaneous activity. It is only when concentrations of 5 to

10 per cent of cocaine are employed that one is able to abolish, reversibly to be sure, the electrical activity (Fig. 4E–F). It is known that cocainization for 5 to 10 min. with 2 per cent solutions is sufficient to abolish almost completely the spontaneous activity of the area striata in the cat (Claes, 1939).

The oscillogram of the turtle is thus an encephalogram, and not a corticogram, as with the use of this method of leading in mammals. The intimate spatial relations between the cortex and the subcortical centers are the essential cause of this difference. We have nevertheless studied the behavior

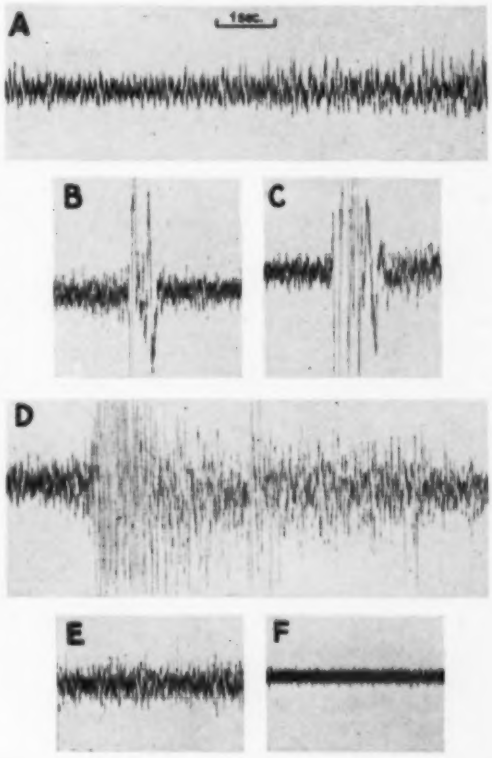

FIG. 4. The local action of strychnine and cocaine on the electroencephalogram of the unanesthetized turtle. 100 μV—100 mm. in the original. 2/3×.

A. Normal activity.

B and C. One minute after the end of a local application of strychnine sulfate 1/1000 (duration of application 2 min.). Note the violent discharges which were unaccompanied by motor manifestations.

D. Immediately after B and C. Bursts of increased electrical activity without convulsive characteristics. The discharges and bursts of activity alternate in the oscillogram.

E. After 2 min. of local application of 2 per cent cocaine. The basal electrical activity remains, although both manifestations of strychnine action have disappeared.

F. After 2 min. of local application of 5 per cent cocaine. Note the disappearance of all electrical activity. This effect is reversible; after some time the activity reappears.

of the electroencephalogram of the turtle in relation to the corticogram or mammals in different experimental conditions. Strychnine in solution of 1/1000, applied to the cerebral cortex of mammals produces characteristically large, rapid, electrical potentials, the so-called "strychnine spikes" of Kornmüller (1935, 1937 see ref. 31), Gozzano (1935), Bremer (1935). This drug applied to the cortex of the turtle acts in an entirely analogous fashion. After these typical strychnine pulsations there was at times a considerable after-discharge lasting 3 or 4 sec., which gradually decreased in amplitude. It seems as if the neurons were not exhausted after the convulsive discharge, but continued to discharge more or less asynchronously. In the mammalian corticogram this phenomenon is not generally observed after convulsive discharges produced by strychnine or epileptic in character. In

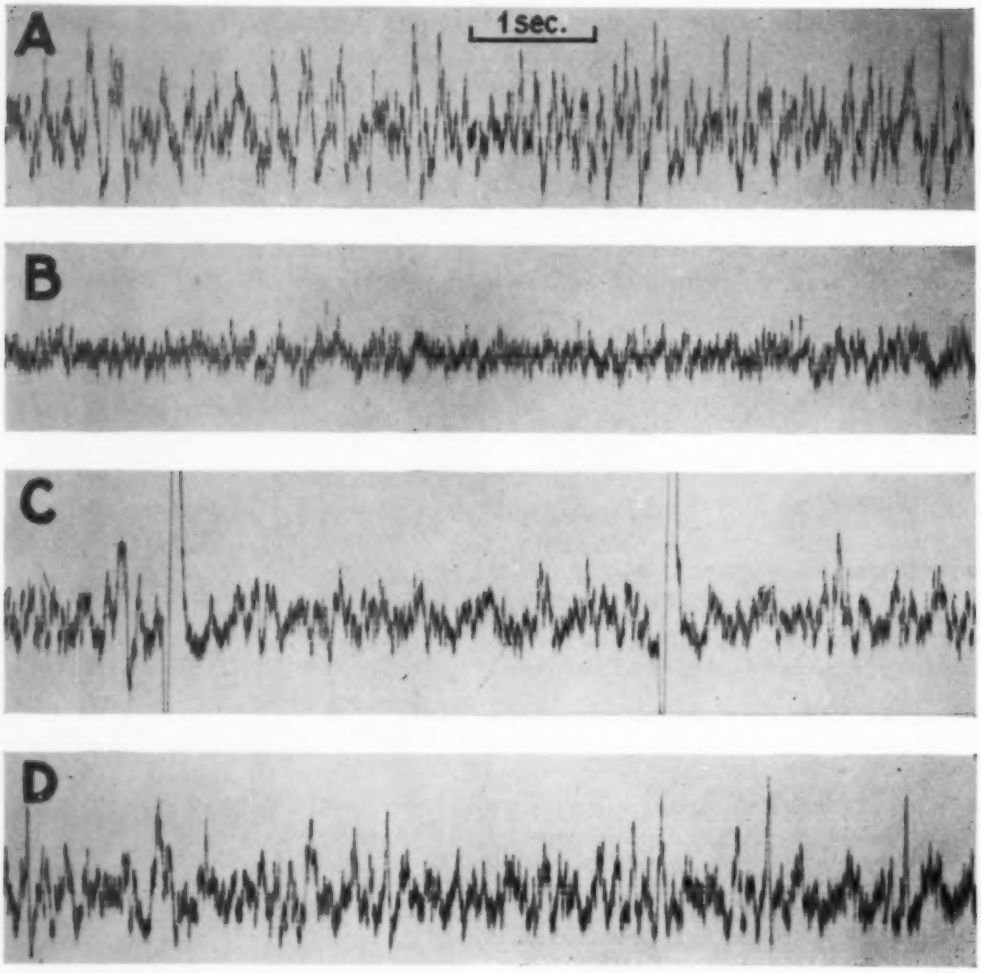

FIG. 5. The local action of strychnine and cocaine on the electroencephalogram of the pigeon. 100μV—15 mm. in the original. No reduction.

A. Normal activity.

B. 30 sec. after the local application of strychnine 1/1000 for 1 minute. Note the diminution of amplitude of the waves and an increase in their frequency.

C. 3 minutes later. Note the "strychnine spikes."

D. Between C and D 2 per cent cocaine was applied locally for 10 sec. Note the disappearance of strychnine discharges, but conservation of the spontaneous activity.

consequence, the question arises whether in the turtle this activity, which we have called "after-discharge" may not in reality be a response of subcortical centers that have been put into activity by the strychnine-induced convulsive discharges of the cortical neurons.

This hypothesis seems to be confirmed by the experimental observations illustrated in Fig. 4. In this experiment the application of 1/1000 strychnine solution to the cortex of the turtle's brain for 1 min. caused the appearance of the typical giant, abrupt discharges, which are altogether analogous to those in mammals (Fig. 5B–C). Some seconds later, one witnessed the ap-

pearance of bursts of activity of great intensity lasting about 5 sec., but without convulsive characteristics. This increase in activity is an augmentation pure and simple of the frequency and amplitude of the normal waves (Fig. 5D). These bursts of activity and convulsive strychnine discharges ("strychnine spikes") alternate in the oscillogram. The local application of 2 per cent cocaine for 2 min. caused the complete disappearance of the strychnine discharges. After an interval the other bursts of activity gradually disappeared also. But the basal activity persisted despite the cocainization (Fig. 4E). Only a 5 per cent solution applied for 2 min. finally resulted in the complete disappearance of activity (Fig. 4F).

Continuing our research, the effects of faradic stimulation on the electroencephalogram of the turtle were examined, a study that has previously been made on mammals. According to Dusser de Barenne and McCulloch (1937), a faradic stimulation is followed by a phase of depression in the spontaneous activity at the point of stimulation. This depression is difficult to explain, because it is simultaneous with the period of primary facilitation which, at least in part, is a cortical phenomenon. In reality the observations of Dow (1938) on the cerebellum of the cat, and of Moruzzi (1938) on the masticator cortex of the rabbit, show that, allowing for an interval for stabilization of the amplifiers, an augmentation of electrical activity in the area that has been stimulated takes place immediately after a faradic stimulation. It is true that at times there is a depression of activity under certain conditions, and in the case of the cerebellum, depression always follows the phase of augmentation (Dow, 1938). Furthermore, an apparent depression of activity may be characterized by a diminution of the amplitude, with an increase in the frequency of the waves, a condition probably produced by the desynchronization of the neurons (Moruzzi, 1938).

In the turtle, faradization of the cortex was followed by a definite after-discharge, which, no matter how strong the stimulus, was never epileptiform in character. In this regard it resembled more closely the after-discharge from stimulation of the cerebellum than of the cerebral cortex in mammals. It is needless to emphasize that this after-discharge may not be, and probably is not, exclusively cortical. We have attempted, although unsuccessfully, to obtain from the cortex of the turtle the effects of "on" and "off" seen in the area striata of mammals with optic stimulation.

Pigeon. The general aspect of the oscillogram derived from the cerebral cortex of the pigeon (Fig. 5) is not fundamentally different from that of the rabbit. The irregular alteration of the large alpha waves with the small frequent waves of the beta type is seen in the bird, but the amplitude of the pulsations is much smaller. As in the case of the turtle, and probably for the same causes, in the pigeon there is simultaneous appearance of electrical activity of the subcortical centers. In consequence, we have an electroencephalogram, and not an electrocorticogram as in mammals. The spontaneous activity was not abolished by superficial cocainization, even though we have shown the extreme sensitivity of the excitability of the cortex to cocaine to be greater than in the case of mammals. It is improbable

and counter to all that we know on this point, that a profound depression of excitability such as this should be without effect on the spontaneous activity. One must then again turn to the conclusion reached in the case of the turtle, that under these circumstances of derivation, subcortical as well as cortical potentials are being recorded.

The action of strychnine in the pigeon is analogous to that in mammals. It is characterized by abrupt, ample, electrical potentials, the so-called "strychnine spikes." Immediately after the local application of strychnine we observed, in accord with what has been described for mammals (Bremer, 1936), an intensification of the preexisting spontaneous activity, without modification of its form. At times this intensification was masked by a diminution of amplitude that results from the desynchronization of the neurons and was then manifest only by a great augmentation of frequency (Fig. 5B). To this phase was quickly added that of the strychnine pulsations—abrupt, ample, and typically spike-like (Fig. 5C). As in the turtle, these strychnine pulsations in all probability are the expression of the activity of the cortex, or at least of the superficial layers of the cerebral hemispheres. The application of 2 per cent cocaine for only 10 sec. caused their immediate disappearance (Fig. 5D). This light cocainization had no important effect on the basic spontaneous activity.

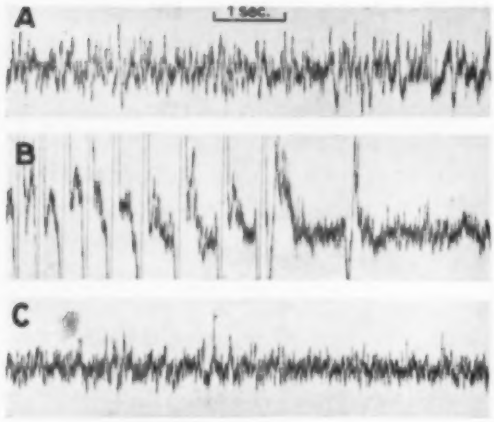

FIG. 6. Epileptic activity of the cerebral cortex produced by faradic stimulation at the site of derivation in the unanesthetized pigeon. 100 μV—15 mm. in the original. ×5/6.

A. Normal activity.

B. Immediately after intense faradic stimulation for 5 sec. Note the violent epileptic discharges.

C. Immediately after B. The epileptic activity has disappeared. The waves are slightly less in amplitude and slightly more frequent than in A.

The action of faradization of the brain on the electrical activity varied with the intensity of the stimulus. With weak stimulation it was difficult to produce an after-discharge of sufficient duration to be seen after stabilization of the amplifiers at the end of the stimulus. If a more intense stimulation was given, there was seen an after-discharge characterized by large waves of an epileptiform nature (Fig. 6). This after-discharge was of brief duration and at times was followed by a depression of the spontaneous activity. We have never been able to observe the phenomenon, so frequent in the rabbit (Moruzzi, 1938a), of the transformation of this after-discharge into a prolonged Jacksonian epileptic attack. Knowing the great development of the optic system in the bird and the important connections of this system to the telencephalon, we have studied with particular interest the

cortical reactions to illumination of the contralateral eye. We were able to obtain the "on" effect regularly, but consistently unable to obtain a clear "off" effect. The effects did not disappear after superficial cocainization of a duration (15 sec.) which suffices completely to abolish the motor responses. Only a prolonged cocainization was sufficient to abolish these effects. It has been shown by Claes (1939) that the effects "on" and "off" in the area striata of the cat are less susceptible to the superficial action of cocaine than is the spontaneous activity. Moreover, spontaneous activity in general is more sensitive to depressing agents than is provoked activity (Bonnet and Bremer, 1937; Moruzzi, 1938 b and c). Perhaps these facts may be used to explain the observations made on the pigeon. However, because of the presence of subcortical centers immediately subjacent, we must not forget another hypothesis, namely, that the effects observed are not cortical in nature, but entirely subcortical. We have no experimental data which might serve to decide this question one way or the other.

Summary

1. The cerebral cortex of the turtle, *Emys europea*, appears to be electrically inexcitable in the usual sense of the term, *i.e.*, there is an absence of visible motor reaction attributable to the excitation of cortical neurons.

2. The electrical activity of low voltage which it is possible to record from the cerebral cortex of the unanesthetized turtle is essentially subcortical (striatal) in origin. However, the ability to produce "strychnine spikes," which are rapidly abolished by superficial cocainization, suggests the existence of a cortical component.

3. The cerebral cortex of the pigeon is electrically excitable by a weak current. The reactions to a unilateral stimulation consist in a conjugate deviation of the head and eyes toward the opposite side, accompanied by a myosis and opening of the palpebral fissure. This response is the expression of the excitation of neurons of the cortical layers, because: (i) the response is abolished almost instantaneously by the superficial cocainization of the excited region; and (ii) an animal which presented a congenital aplasia of the cortex on one side, as verified histologically, did not react to the application of strong stimulation applied to this side, while the opposite cortex which appeared to be normal gave the usual response to weak stimulation. These conclusions concerning the reality of motor reaction dependent upon the cerebral cortex of birds confirm and justify by new evidence the studies made by older workers, particularly those of Ferrier and Kalischer. The negative results reported more recently are possibly explained by the use of light anesthesia or by the inhibition resulting from failure to apply cocaine to the borders of the wound in the unanesthetized animal.

3. The simultaneous excitation of two symmetrical points on the right and the left cortex of the pigeon with currents of equal intensity results in rhythmic movements of the head in the vertical plane, suggestive of pecking movements in the intact animal.

4. The spontaneous electrical activity derived from the cerebral cortex of the unanesthetized pigeon resembles closely that of the rabbit when awake. The partial resistance of this activity to superficial cocainization indicates that it includes a subcortical component. The superficial strychninization of the cortex causes the appearance of the large "strychnine spikes" which are abolished rapidly by superficial cocainization. A brief cortical faradization releases a short after-discharge of epileptiform type.

5. The cerebral cortex of the pigeon reacts to the illumination of the contralateral eye by a large initial wave (effect "on"). The cessation of the illumination does not provoke a distinct "off" effect.

6. The general conclusion derived from an analysis of the excitability and the electrical activity of the cerebral "cortex" of the turtle and the pigeon indicates that, in the pigeon at least, the superficial layers of neurons covering the striatum dorsally and posteriorly have physiological properties and a functional significance—the latter being essentially opto-kinetic—much like those of the neo-pallium of mammals.

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STUDIES IN THE PHYSIOLOGY OF THE EMBRYONIC NERVOUS SYSTEM:

IV. DEVELOPMENT OF ACETYLCHOLINE IN THE CHICK EMBRYO*

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INTRODUCTION

THAT ACETYLCHOLINE (ACh) is liberated when cholinergic nerves are stimulated seems well established. Recent evidence suggests that ACh is also liberated at central synapses and at neuromuscular junctions of striated muscle, but it is still unsettled whether liberation of ACh by electrical stimulation is a coincidence—*i.e.*, merely a product of increased metabolic activities of nervous tissues possibly associated with pathological processes (Fleisch *et al.*, 1936; Lorente de Nó, 1938)—or whether the presence of such a chemical substance is actually concerned with transmission of impulses at synapses and at neuromuscular junctions. Study of the origin and development of ACh in embryos, and its correlation with anatomical development of the nervous system and of reflex activities may throw light on the question. The chick embryo appears to offer favorable material for this purpose, since information is already available concerning the development of its nervous system and since the development of the chick's reflex movements is known (Kuo, 1938).

MATERIALS AND METHODS

Chick embryos of 2 to 12 days were used. Embryos of a given age were collected and placed in a dish containing eserinized standard Ringer's solution. The embryos were quickly cut into small pieces and thoroughly ground. The ground mixture was immediately tested for ACh. In those experiments for determination of ACh, the concentration of eserine varied, and no attempt was made to dilute the mixture in proportion to weight of embryo. In identification experiments only mixtures from 3-, 4-, and 5-day embryos were tested. Tests were made on frogs' hearts (Straub method), frogs' rectus abdominis muscles (Chang and Gaddum, 1933), and on dorsal muscles of leeches (about 15 segments). Both frog's rectus and leech muscle were bathed in 2 cc. of eserinized Ringer's solution, the concentration of eserine being 1: 300,000. However, in those experiments conducted to estimate the quantitative variations of ACh from day to day during embryonic development, the following procedure was rigidly followed: (i) Only frog's rectus abdominis was used. (ii) The concentration of eserine in the bathing fluid was 1: 300,000. (iii) The concentration of eserine contained in the embryonic mixture was 1: 50,000, since higher concentrations of eserine gave better ACh yields. (iv) The embryos were weighed before they were cut and ground. (v) The mixtures were diluted with Ringer's solution in proportion to the weights of the embryonic tissues, so that equal weights of embryonic tissues of different ages would tend to have equal volumes of mixture (Table 1). (vi) All tests were made immediately after the tissues were ground, since standing tended to increase the ACh in the mixture.

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Results

The identification of ACh. With the exception of the paper of Youngstrom (1938) there has been no previous report in the literature of a systematic investigation of the developmental history of ACh in the embryo. Hence it seemed desirable to employ every existing physiological method for the certain identification of the ester. All six methods proposed by Chang and Gaddum (1933) were used, and all results were positive and consistent. Figures 1 and 2 give samples of results. It can be concluded that the substance assayed in the embryonic mixture was ACh.

Fig. 1. Responses of frog's rectus to eserinized embryonic (chick) extract and to plain extract. F-4 illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, and E_1 the response to 1 cc. of plain embryonic extract of 4 days of incubation.

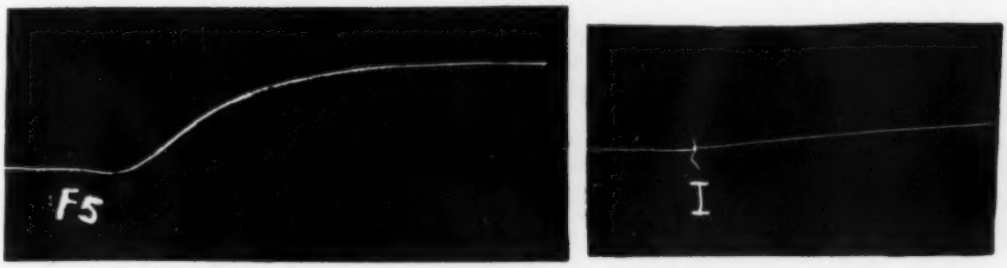

Fig. 2. Responses of frog's rectus to eserinized embryonic (chick) extract treated with weak acid and with weak alkali. F-5 illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, to which 0.1-normal HCl (about 4 drops per cc.) was added. I illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, to which 0.1-normal NaOH (about 4 drops per cc.) was added. In both cases after HCl or NaOH was added the extract was boiled, cooled, neutralized, and tested with litmus paper before being assayed on the rectus.

Quantitative changes of ACh during embryonic development. Table 1 gives the quantitative estimates of daily variations of ACh during embryonic development. The tests were made with embryos of 2 to 12 days, inclusive. The table reveals that ACh can be detected in the embryonic tissues as early as $2\frac{1}{2}$ days. It is highly probable that if sufficient embryos younger than $2\frac{1}{2}$ days had been available, it would have been possible to detect ACh in the embryo of 2 days or even younger. There is a great increase in the amount of ACh per embryo from day to day during incubation. Such in-

crease is more apparent than real, however, for there is no significant and regular increase or decrease per unit of weight from the fourth to the twelfth day of incubation (Table 1, last column). The broken-line curve in Fig. 3

Table 1. Quantitative changes of ACh during the first 12 days of incubation.

Incubation in days	Total number of embryos	Total weight in gm.	Total diluted extract in cc.	Total amount of ACh in γ	Amount of ACh per embryo in γ	Amount of ACh per gram of total weight in γ
2	484	2.18	5	0.00	0.0000	0.00
2.5	468	6.16	15	2.04	0.0044	0.33
3	480	8.21	20	3.41	0.0071	0.42
4	120	7.92	20	6.18	0.0515	0.78
5	44	7.91	20	7.44	0.1691	0.94
6	18	8.28	20	6.32	0.3511	0.76
7	11	8.21	20	7.27	0.6609	0.89
8	10	12.18	30	9.50	0.9500	0.78
9	10	16.21	40	15.60	1.5600	0.96
10	10	23.81	60	21.11	2.1110	0.89
11	8	26.72	65	24.05	3.0063	0.90
12	6	27.81	70	22.47	3.7450	0.81

(page 492) shows more clearly that between the fourth and the twelfth day the production of ACh per gram of tissue fluctuates irregularly and that the quantity of daily variation during this period is relatively small.

Correlation with development of nervous system

A search of the literature dealing with the morphological development of the chick's nervous system revealed one fact clearly: *i.e., no synapse is found in any part of the nervous system up to the end of the third day of incubation*. Although fibrillogenesis in the central nervous system begins as early as the 38th to the 42nd hour of incubation (Cowdry, 1914; Tello, 1923; Windle and Austin, 1936), the fibers are short and few, even in the 3-day embryo, and there is no indication of the appearance of synapses. Comparably, primary sympathetic trunks do not appear until the end of the third day or the beginning of the fourth, and the *anlagen* of the secondary sympathetic trunks do not arise earlier than the sixth day (His, 1897; Abel, 1910; Kuntz, 1910). When the primary sympathetic trunks first appear, moreover, they are merely aggregates of cells.

ACh thus appears long before any synapses are formed and at a time when the bulk of the neural tube is still made up of epithelial cells and germinal cells not yet differentiated into primitive nerve cells, when neuroblasts are still relatively few, and when neuro-fibers are still short and few. Furthermore, as development proceeds, the length and number of neuro-fibers, synapses and neuromuscular junctions increase from day to day, whereas the relative amount of nervous tissue in proportion to other tissues decreases rapidly. In the early stages, brain size is enormous in proportion to other parts of the body, and the head is extremely heavy. But the ratio of head

weight to total body weight falls from 67 per cent on the 4th day to 30 per cent on the 12th, and to 18 per cent on the 20th (Kuo, 1932b). However, in spite of these progressive morphological changes, the increase of ACh per gram of tissue from the 4th to the 12th day of incubation is so irregular and uncertain that there is no apparent correlation between the development of ACh and the development of the nervous system (Fig. 3, broken-line curve).[1]

Correlation with development of reflexes

In the chick no movements, spontaneous or in response to reflex stimulation, can be observed before the 4th day of incubation, and reflex activities increase rapidly in kind, magnitude, and frequency per minute during the first half of incubation (Kuo, 1932a, 1938). Table 2, giving average frequency of spontaneous reflex movements per minute during incubation, is based on data accumulated by the writer during recent years. Comparison of this table with the last vertical column of Table 1 shows that there is no indication of a correlation between the development of ACh and the development of reflexes during the period studied; ACh appears long before reflex movements and then shows an irregular fluctuation from the 4th day on, whereas reflex movements increase rapidly and regularly from the 4th to the 8th day and then steadily decrease. This lack of correlation is illustrated more clearly by Fig. 3.

Table 2. Average frequency of spontaneous reflex movements per minute in the chick during embryonic development.

Days of incubation	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Reflex movements	0.0	2.1	4.8	7.8	11.6	12.3	11.1	7.4	4.8	4.6	3.9	3.4	3.8	3.6	3.5	3.1	2.8	3.4

Discussion

In view of such discrepancies between anatomical maturation and the concentration of ACh in relation to the appearance of reflexes, one is tempted to conclude that ACh, as it is found in a mixture of embryonic tissues, is not concerned with transmission of nerve impulse in the embryo. Such a conclusion does not preclude the possibility that the choline ester liberated at a particular moment of nervous excitation may act as a transmitter at synapses or at neuromuscular junctions. Such a possibility, however, remains to be proved. The mere presence of ACh or even an increase in

[1] The results and interpretations of the investigation by Nachmansohn (Thomas and Nachmansohn, 1938; Nachmansohn, 1938, 1939) on the development of choline esterase in the chick embryo are of uncertain significance, since he appeared unfamiliar with the developmental history of reflex movements (Kuo, 1932a, 1938) and did not begin his investigation until the incubation period was nearly half over (9 days). Moreover, his data are incomplete during the second half of incubation, since he used only 9-, 12-, 16-, and 20-day embryos. The amount of tissue he used for his tests was probably too small to warrant conclusions.

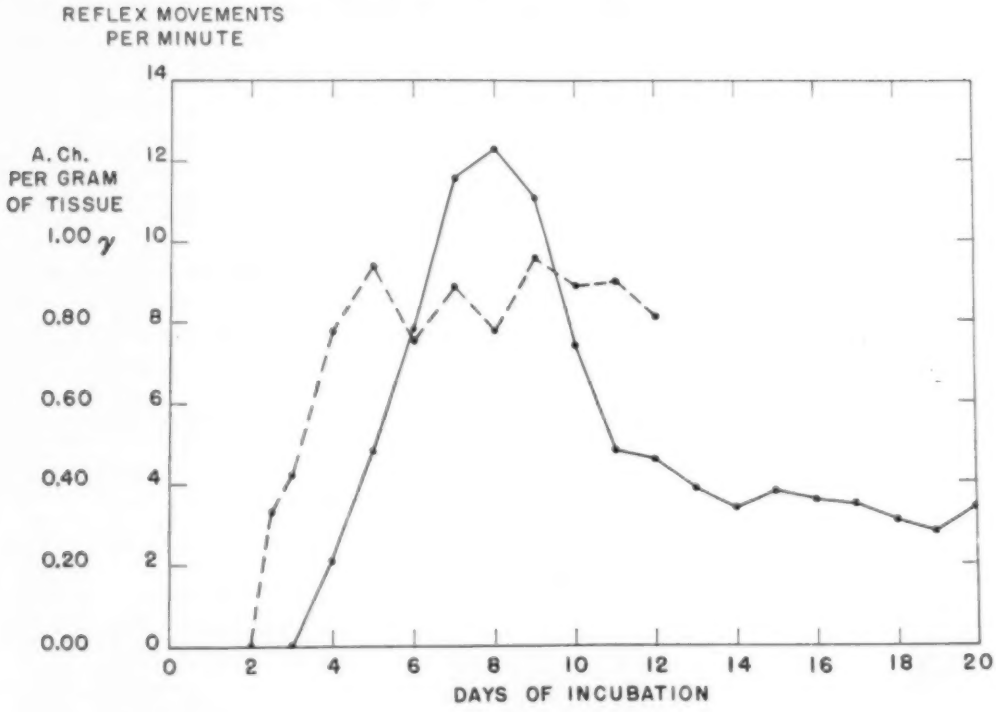

FIG. 3. Comparison of development of ACh with development of reflex activities in the chick embryo. The full-line curve shows the average spontaneous reflex movements per minute and the broken-line curve the daily variations in ACh per gm. of tissue during embryonic development. The curves are plotted from the data in Tables 1 and 2.

its output at nerve terminals or synapses at the time of excitation is no sure evidence that the ester is actually concerned in transmission. The fact that ACh exists in most animal tissues, including such nerveless structure as human placenta, and can be detected when hydrolysis of the substance is prevented, has not been given satisfactory explanation by the proponents of chemical transmission.

The present results create a more difficult drawback, which can not be lightly dismissed as irrelevant to the theory. They lend support to the demand for better evidence that accumulation of ACh is not merely a result of increased metabolic activity or injury or other pathological process. The claims made by MacIntosh (1938a, b) and by Bacq and Coppée (1938), that in degeneration experiments there is a time at which both the preganglionic fibers and ganglion cells appear to be still functionally intact whereas transmission across the synapses no longer occurs (which event coincides with the disappearance of ACh from the ganglion), are important in this connection, but they must be more thoroughly and systematically reinvestigated before they can be fully evaluated. The view that liberation of ACh is a general phenomenon associated with metabolic activities and is not in any way peculiar to nervous excitation also lacks decisive proof.

The results of Lorente de Nó (1938) are significant but not conclusive and have been contradicted by a more recent investigation (MacIntosh, 1938b). The evidence from the chick embryo here reported strengthens the metabolistic point of view but does not disprove the transmission theory.

Summary

1. Tissues of chick embryos were ground and assayed for acetylcholine (ACh).

2. ACh increases from $2\frac{1}{2}$ to 4 days of incubation and then shows random fluctuations until the 12th day.

3. There was no indication of a positive correlation between the development of ACh in the chick and the development of reflexes and of the nervous system.

4. The bearing of the results on the theory of chemical transmission is discussed.

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EFFECTS OF ACOUSTIC STIMULI ON THE WAKING HUMAN BRAIN*

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INTRODUCTION

SPECIFIC immediate electrical responses of the cerebral cortex to peripheral stimulation have been described for the anesthetized and the unanesthetized animal (see References for principal citations). More general and persistent modifications of pre-existing activity, such as the initiation or the checking of fast frequencies are also familiar (Bartley and Heinbecker, 1938; Bremer, 1937a, b, c). In the human, the checking of the alpha rhythm by opening the eyes, and, less regularly, by a sudden sound, was described by Berger (1929) and widely confirmed by others (see References). But immediate positive responses in the human to sounds or other stimuli have received only scant attention, partly because they do not appear under all conditions and partly because observers have feared being misled by artefacts due to muscular movement and particularly movements of the eyes (cf. Rohracher, 1938; Travis, Knott, and Griffith, 1937; and Wessell and Carmichael, 1938).

The present observations, made in 1935–36, now take on a special interest because of their close relationship to the responses of the human brain during sleep which are described in another paper (Davis, Davis, Loomis, Harvey, and Hobart, 1939). They were obtained in 41 experiments on 38 adult men and women.

METHODS

A single-channel Grass amplifier with an "undulator" type of ink-writer (Garceau and Davis, 1935) was employed. Our silver-silver chloride electrodes were approximately 5 mm. in diameter. Sanborn "Redux" electrode paste was rubbed into the scalp, and collodion was used to hold the electrodes in place. Reference electrodes made like ear rings were applied to both ear-lobes, and connected in parallel. Standard placements on the head were the vertex and the occiput 2 cm. above the inion in the mid-line. A few experiments included, in addition, the mid-frontal region, 6 cm. on either side of this region, and the temporal areas.

A Clough-Brengle beat-frequency oscillator and a loud-speaker were the source of the sound. A signal recorded automatically on the tape the onset and duration of the stimulus. Both loud and faint tones in the range from 250 to 2000 cycles were employed. Standardization of experimental conditions included a consideration of the subject's physical comfort and his physiological state of alertness. This requires that the subject be free from fatigue or drowsiness before the experiment starts. Careful explanation of the purpose and each step of the procedure were given so that the subject would understand and be free from apprehension.

Procedure. While the subject lay comfortably on a bed in the softly lighted room, he was asked to open and close his eyes several times. The purpose of this was to have an electroencephalographic record of the subject's eye-movements, his responses to light and to the sound of the experimenter's voice when asked to open and close his eyes. The

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sequence of tones was 250, 500, 1000, and 2000 cycles, each being given twice, first as a faint, then as a loud tone. The duration of each tone was a few seconds and the interval between tones a longer period. The sequence was then reversed. Finally, the subject was told that the sound stimuli were going to be changed and put in irregularly without any preconceived arrangement. This was done.

Results

Electrical responses to sound stimuli cannot always be detected. When observed they are of the same character regardless of the area in which they appear. The response was seen more clearly from the occiput than from the temporal or frontal areas, but most prominently from the vertex (Fig. 1).

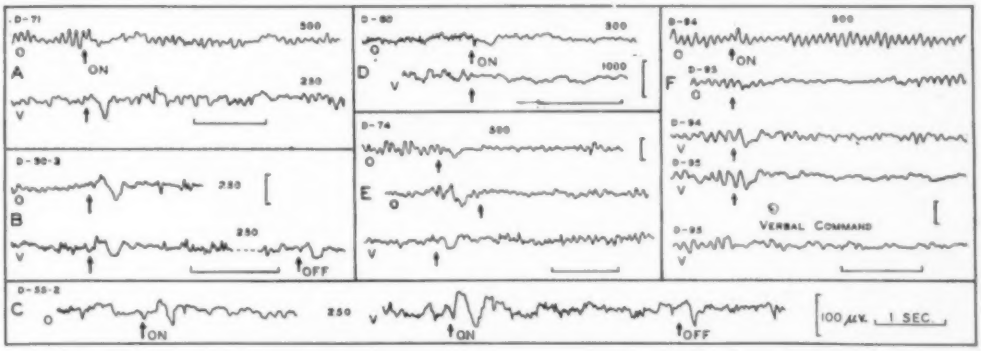

Fig. 1. On-effects and modifications of spontaneous rhythms in response to sounds. The frequency employed is indicated in each case. No measurements of loudness were made. O = monopolar occipital record, V = monopolar record from vertex. Reference electrodes on ear-lobes connected in parallel. Paired tracings do *not* represent simultaneous records. Calibrations are for 1 sec. and 100 μV. throughout.

A—checking of alpha rhythm and on-effect in an alpha subject

B—on- and off-effects in a non-alpha subject. Note also checking of beta waves in vertex record

C—same as B in another subject

D—checking of fast (beta) frequencies in another non-alpha subject

E—typical on-effects. "Anticipatory" reaction in second line

F—checking of alpha rhythm and on-effects in a pair of identical twins, D-94 and D-95; also effect of a verbal command.

The response was an "on-effect" composed of a diphasic, and sometimes triphasic, wave. During the first phase the active electrode becomes electrically more negative. The total duration of the on-effect was approximately 0.3 sec. or less. The voltage, measured from the peak of the negative phase to the trough of the positive phase, ranged from 100 μV. to deflections which were just distinguishable from the background of physiological activity. The latencies could not be precisely measured but were of the order of 30 to 40 msec. (see Fig. 1).

An "off-effect" sometimes occurred upon cessation of the tone (see Fig. 1B and C), and was similar in character to the on-effect (Fig. 1B and C). It did not occur as often as the on-effect, but sometimes appeared upon cessation of a stimulus which produced no visible on-effect. It was rarely as prominent as the on-effect.

On-effects appear in both the alpha and the non-alpha type of individuals (Fig. 1, A and F, B and D). In non-alpha subjects, the on-effect can be seen more easily due to the lack of alpha rhythm. In alpha subjects, the only response to sound often appears to be a momentary checking of the alpha rhythm (Fig. 1, A 1st line, and E 1st line). When the speed of the tape is doubled, the checking of the alpha rhythm can be studied in greater detail. The wave-length and shape of the alpha rhythm is somewhat similar to that of the on-effects. The phase-relations of the alpha wave and the on-effect may be such that the on-effect is obscured. The alpha wave is often distorted when checked by sound stimuli. An alpha individual usually has less alpha rhythm in the vertex and frontal areas than in the occiput. Wherever the alpha is, it is distinguished from other frequencies by being modified when eyes are opened or closed under standard conditions. The alpha in Fig. 1F is the 11-per-sec. rhythm (1st 2 lines). As usual, the on-effect on the occiput record is not as clearly demonstrated as in the vertex, where a 9-per-sec. rhythm is emerging from an alpha rhythm, and is abruptly stopped.

In the non-alpha group, two subjects had cortical patterns which were composed of predominantly fast frequencies. The only response to acoustic stimuli was a checking of the fast frequencies which followed every stimulus (Fig. 1D, 1st line). In another subject fast frequencies appeared upon and during stimulation. In still other non-alpha subjects, marked on- and off-effects were produced without interruptions of the fast frequencies.

Under standard conditions, as briefly defined above, responses to sound stimuli take place in a variable manner. The variability does not appear to depend upon the loudness or on the frequency in the range of tones given. The loud tones were not intense enough to produce a startle reaction. The alpha and non-alpha subjects both show variability of response in the same way. When standard conditions were altered, responses to sound stimuli were also modified. If a subject is made uncertain of the procedure, his "psychological set" is modified (cf. Durup and Fessard, 1935; Knott, 1939; Bakes, 1939). When even an orderly sequence of sounds was given without warning, on-effects often eventually appeared before the stimuli were given. These are interpreted as "anticipatory" on-effects (Fig. 1E, 2nd line). The "psychological set" causes the subject to "anticipate" the stimulus which he thinks may be given.

Some individuals show this anticipation only when random frequencies or irregular time intervals are unexpectedly given. Others would respond with an "anticipatory" on-effect if a series of regular sound sequences were cut short. If the usual duration of the sound was cut short, an off-effect would appear at the time of the usual cessation of stimuli. If it was prolonged, the off-effect occurred before the tone ceased. In other words, many "anticipatory" responses have been produced by altering the frequency and time sequences, but were best brought about by causing the subject himself to be uncertain of the procedure as a whole. The character of the responses, however, did not change.

If the physiological condition of the subject becomes modified as he shifts from the alert state to sleep, and the "psychological set" is maintained, the on-effect becomes more pronounced and more predictable. Contrasting this effect on the responses to sound with the responses of the alpha rhythm to light, four stages may be described as shown in Table 1.

Table 1

	α Rhythm (Eyes Closed, No Sound)	Change in α Rhythm on Opening Eyes	On-Effect to Sound
1. Alertness	present in usual degree	α checks sharply	α check and/or on-effect or no visible effect
2. α-optimum state	maximum for the individual	α check not so clean-cut	on-effects more frequent and definite
3. Drowsiness	reduced and variable, fades out	α rhythm returns (a reversal of stage 1)	higher voltage on-effect
4. Sleep	absent	—	on-effect with return of fast waves [*K*-complex (see Davis, Davis, Loomis, Harvey, and Hobart, 1939)]

Discussion

A sound-proof room was not used for these experiments because it had previously been observed that people coming into a sound-proof room elsewhere in our laboratories were profoundly affected by the unnatural quiet and the unusual sound of their own voices in such a room. One person remarked that "the utter stillness was a violent stimulus in itself." From these observations, it was felt that the normal state of the individual could best be tested in the electrically shielded, quiet but not sound-proof room. Our auditory stimulation was carried out with eyes closed, which greatly reduced the possibility of eye-blinks or -movements obscuring the on-effects. The distribution, relative magnitude and the shape of the on-effect usually differentiates it clearly from eye artefacts.

Numerous on-effects or "evoked potentials" have been described in animal experiments. It is therefore not surprising to find an on-effect to auditory stimulation in the human cortex. The unexpected feature is rather its diffuse character and the variable conditions of its appearance. Obviously it does not represent the first arrival of afferent impulses in the auditory projection area. It might be thought to correspond to the response of cortical neurons to the first sensory influx, which is apparently the nature of many evoked potentials (Fischer, 1932; Kornmüller, 1937; Bremer, 1937a, b, c; Bishop and O'Leary, 1936). But, according to the analysis by Bartley, O'Leary, and Bishop (1937) of the responses of the optic cortex at least two types of secondary reaction must be recognized, one of short latency, fairly well localized to the immediate sensory projection area, enhanced by strych-

nine and depressed by narcosis, and another slower response of longer latency, spreading more widely through the cortex, depressed by strychnine and resistant to moderate narcosis. It is to this class, which apparently includes the widespread secondary discharge which is enhanced by deep barbiturate narcosis (Derbyshire, Rempel, Forbes, and Lambert, 1936; Forbes and Morison, 1939) and also some of the auditory responses of Bremer, that our present on-effect most probably belongs. The increasing prominence and certainty of appearance of our on-effect with drowsiness (see also Davis, Davis, Loomis, Harvey, and Hobart, 1939) supports this tentative identification, as barbiturate narcosis corresponds rather closely to natural sleep (Bremer, 1937c). The variable conditions of appearance, including the "psychological set" seem to depend on the pre-existing activity or "tone" of the cortex or of the subcortical structures involved in the wide diffusion of the on-effect. The various other secondary effects upon the alpha rhythm and faster frequencies may be interpreted in similar fashion, but our facts are too scanty to warrant further speculation.

Conclusion

Acoustic stimuli cause electrical on-effects and off-effects in the waking human brain. Though tones did not always produce visible responses, there appeared to be no difference between alpha and non-alpha types of subjects.

The on-effect, composed of a diphasic and sometimes triphasic wave, was most prominent at the vertex. The first phase was negative. Its latency was about 30 to 40 msec. The total duration of the on-effect was approximately 0.3 sec. or less. The voltage measured from peak to trough ranged from just visible to 100 μV. Frequently there was an off-effect similar to the on-effect, but never as prominent.

A checking of the alpha rhythm was sometimes the only visible response. Fast frequencies were checked in two non-alpha subjects, and caused to appear in a third.

"Anticipatory" on-effects or off-effects appeared at an appropriate interval when a regularly spaced sequence of tones was unexpectedly stopped or prolonged. If a subject was not aware that random sequences of different tones were to be given, or that regular sequences were going to follow irregular sequences, the "anticipatory" on- and off-effects would become more unpredictable. If the "psychological set" remained unaltered in relation to the experiment, but the physiological condition progressed from alertness to sleep, the on-effect would always become more predictable and more prominent.

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ELECTRICAL REACTIONS OF THE HUMAN BRAIN TO AUDITORY STIMULATION DURING SLEEP

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INTRODUCTION

THE SPONTANEOUS electrical activity of the human brain has been described both for the waking state and for sleep by many investigators (see Jasper, 1937, Davis, H., 1939, for references). The modifications of electrical activity as a result of peripheral sensory stimulation in the waking state are slight and have received much less attention. A conspicuous effect in many subjects is the "check," or inhibition, of the 10-cycle "alpha" rhythm which occurs when the eyes are opened. Definite "on-effects," particularly in response to sounds, have been mentioned casually by several investigators and described systematically by one of us (Davis, P. A., 1939, *q.v.* for references).

In sleep one reaction to sensory stimulation is a return of the waking pattern; but three of us have described (Loomis, Harvey, and Hobart, 1938) a more specific disturbance pattern which we designated as the "*K*-complex." The *K*-complex and the waking on-effects are of considerable theoretical importance because of the possibility of identifying them with similar responses of the brains of animals, and thereby coördinating the separate fields of human and of animal investigation. We therefore undertook further investigation of the human *K*-complex in an endeavor to analyze it into its components, and to compare the components with other electrical phenomena in the brains of both man and animals.

METHOD

Twenty-five experiments on sleep were carried out at the Loomis Laboratory in 1938 utilizing the six-channel, ink-writing electroencephalograph and its accessories, described in a previous paper (Loomis, Harvey, and Hobart, 1938). The subjects went to bed either for an afternoon nap or for a full night's sleep. Various types of electrodes (Davis, Davis, Loomis, Harvey, and Hobart, 1939) were employed, including silver, solder, and zinc. Our standard placements were: frontal (at the usual hair-line, 6 cm. to right and left of the midline), central (in the frontal plane of the auditory meatuses, 6 cm. to right and left), occipital (2 cm. above the inion and 5 cm. to right and left), temporal (1 cm. above the tip of the pinna of the external ear). In all cases, recording was by the so-called "monopolar" method. Reference electrodes were placed on one or both ear-lobes or on the mastoid region immediately behind the ears.

RESULTS

On-effects to sounds in the waking state

An earlier series of observations by one of us on the waking on-effect are reported separately (Davis, P. A., 1939). The findings were confirmed in the present experiments. In response to the onset of a steady tone, there is usually a definite diphasic response beginning with a negative wave

(latency 50 to 100 msec.) followed by a slower positive wave (see Fig. 1), in addition to the momentary checking of the alpha rhythm (Fig. 4_1). The diphasic response appears to be a true on-effect. It is widely generalized throughout the cortex and has greater voltage in the central and precentral regions than in the occipital or temporal areas.

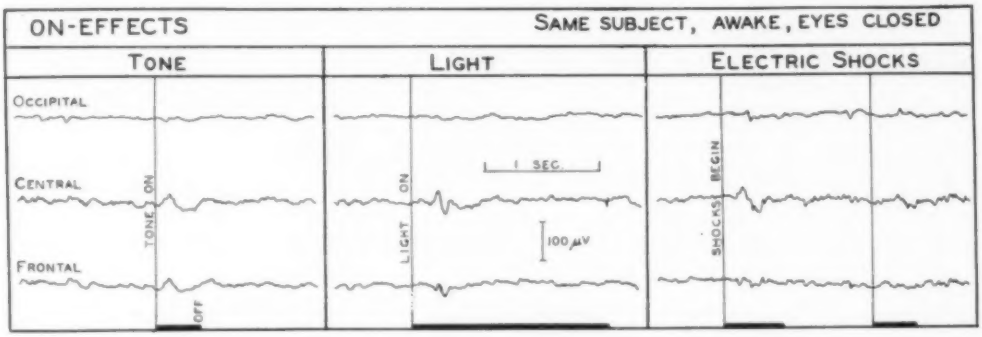

FIG. 1. Waking on-effects to tone of 500 cycles at 70 db above threshold, to indirect illumination of room by a 100-watt incandescent lamp, and to electric shocks delivered to left fourth finger. The subject is a 37-yr.-old man, lying in bed awake, with eyes closed throughout. Noise-level in room at position of sleeper's head, principally from ventilator fan, was 35 to 40 db. Intensity of stimulating tone measured also as a noise-level. Reference electrode on right mastoid region. Scalp electrodes also on right side of head. In this and all subsequent figures an upward deflection represents increasing electrical negativity of the scalp relative to the reference electrode on ear or mastoid region.

It is unnecessary to repeat the detailed description of the auditory on-effect or the conditions favoring its appearance (see Davis, P.A., 1939), but it is significant that neither the check of the alpha rhythm nor the diphasic on-effect is specific for auditory stimulation. Very similar responses have been obtained from visual and also from electrical stimulation. Figure 1 shows clear on-effects from a subject whose interim record was unusually flat. The minor differences in the shape and latency of the on-effects to light (diffuse illumination from a 100-watt bulb seen through closed eye-lids), tone, and electricity (induction shocks to left fourth finger) tended to be characteristic of the particular form of stimulation, but the distribution over the cortex was the same for all three.

The diphasic on-effect and the modification of the alpha rhythm are obviously both of them generalized secondary reactions of the cortex which may be observed under favorable conditions. They should not be interpreted as equivalent to the immediate and localized responses in a particular sensory area which are seen in experiments on the exposed cortex of animals.

The K-complex in sleep

The response which usually follows auditory stimulation during sleep is much larger and much less variable than the waking on-effect. The response is complex, and its characteristics vary systematically with the

stage of sleep. Figure 2 illustrates the *K*-complex as it appears in the *C* stage, recorded simultaneously from six different cortical areas. Shortly after the beginning of the stimulating tone, the scalp becomes electrically negative with respect to the ears by 50 to 100 μV. At about 0.75 sec. the scalp abruptly becomes more positive by 100μV. or more (*S* in Fig. 2). This major positive wave is followed by a slower return to the original electrical level. Fast waves, often sharp and irregular, sometimes in clear and regular 14-per-sec. rhythm and sometimes in slower 8-per-sec. rhythm (as at *F* in

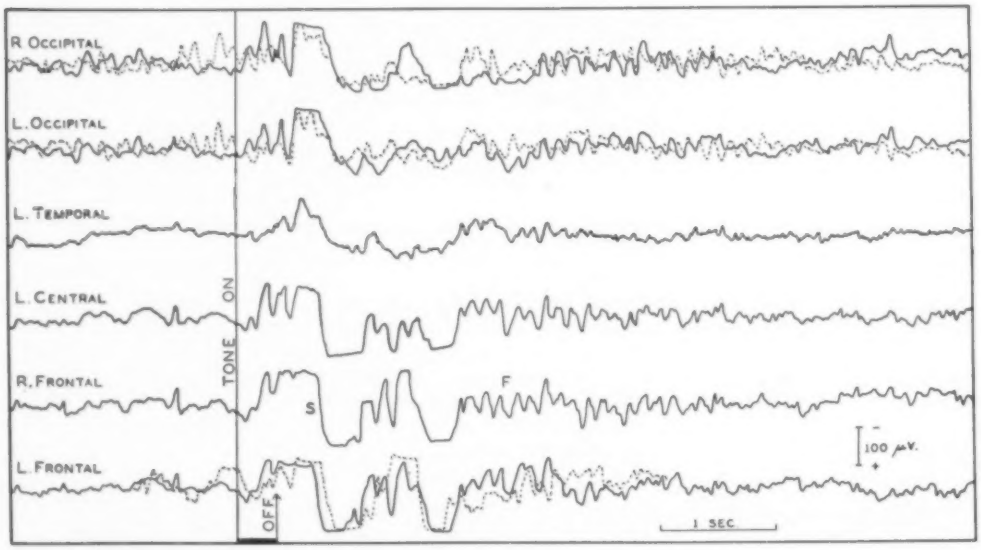

FIG. 2. Typical *K*-complexes in response to tone during *B*-*C* stage of sleep. Afternoon nap, 21-yr.-old man. Tone, 500 cycles at 70 db above threshold. Noise-level as for Fig. 1. Condenser across terminals of loud-speaker to eliminate click at onset of tone in this and subsequent experiments. Reference electrode just above left mastoid. Dotted lines show response at 3 electrodes to a similar stimulus 1.5 min. after the response shown by solid lines. The fast component (*F*) is prominent and is characteristically 8 per sec. in this subject until deep sleep is reached. Note the artificial flattening of the tops and bottoms of the slow waves (*S*) by the current-limiting tube in the output circuit (Loomis, Harvey, and Hobart, 1938).

Fig. 2) are superimposed on the slow waves and may persist for several seconds afterward. The abrupt swing from negative to positive usually occurs at about 0.75 sec., but it may be delayed until more than 1 sec. after the onset of the tone. The first slow negative swing is usually preceded in this subject by waves of medium, that is, 6- to 10-per-sec. frequency. The major features of the pattern are usually closely reproduced in successive trials on the same subject, as illustrated by the dotted lines in Fig. 2. Figure 2 also illustrates a definite tendency of the slow-wave sequence to become rhythmic (note particularly the frontal records). A rhythmic activity of the slow component is highly characteristic of deep sleep (cf. also Fig. $3_{5,6}$).

The distribution of the *K*-complex over the head follows closely the distribution of the waking on-effect. The voltage is regularly greatest in the central and precentral regions, and nearly as great in the frontal. The disturbance is definitely smaller at the occiput and still smaller in the temporal region. The temporal region, however, always gives a low-voltage record for all features (*K*-complexes, on-effects, waking alpha rhythm, etc.), perhaps because of the shunting effect of the soft tissues, notably the temporal muscles, external to the scalp. The *K*-complex may be of higher voltage in the frontal than in the central region, particularly when the major waves are slow and rounded, as in Fig. 4. Very rarely the *K*-complex is most prominent at the occiput.

A characteristic *K*-complex is usually produced by even a rather faint tone (20 db above the noise-level of the room) if the sleeper is in the *B* or *C* stage. The responses are larger and failures of response are fewer if the tone is loud. The pitch of the tone within the range employed (200 to 3000 cycles) is unimportant except that after a series of tests at one pitch, a shift to a new pitch is rather likely to awaken the sleeper. It is possible to initiate typical *K*-complexes by turning on a light in the experimental room or by applying mild electric shocks to the subject's finger, but neither of these stimuli are nearly as effective in evoking *K*-complexes as are sounds.

In one experiment, an effort was made to condition the *K*-complex to electrical stimulation. Electrical stimulation and sound were combined for a number of trials and then the tone was omitted. The response to the electrical stimulation was not clearly greater than it had been previously. There seemed to be some additive effect between the two types of stimulation, as tone plus electrical stimulation gave a somewhat greater proportion of positive responses than did the tone alone. It is difficult to perform satisfactory experiments of this sort, as the responses vary considerably with the depth of sleep and it is difficult to hold the sleeper in a steady state for a long enough time. Usually he either goes too deeply asleep or else, if stimulated too vigorously or too frequently, he awakens.

Spontaneous *K*-complexes are common. Sometimes definite causes can be found for them, the commonest being the sleeper's own breath sounds. It is quite amusing to observe the regular appearance of electrical disturbances with each snore, but it interferes seriously with systematic experimentation. For many *K*-complexes, however, we have found no assignable external cause.

Relation of the K-complex to the stage of sleep

The description of the *K*-complex thus far has been based on the responses of sleepers in the *C* stage of sleep. It is in this stage that the *K*-complex appears most clearly. The genesis of the typical *K*-complex with the onset and progress of sleep is illustrated in Fig. 3. In this experiment the subject (a 14-year-old boy) was instructed to turn off the tone whenever he heard it, by squeezing a rubber bulb placed in his hand. The waking

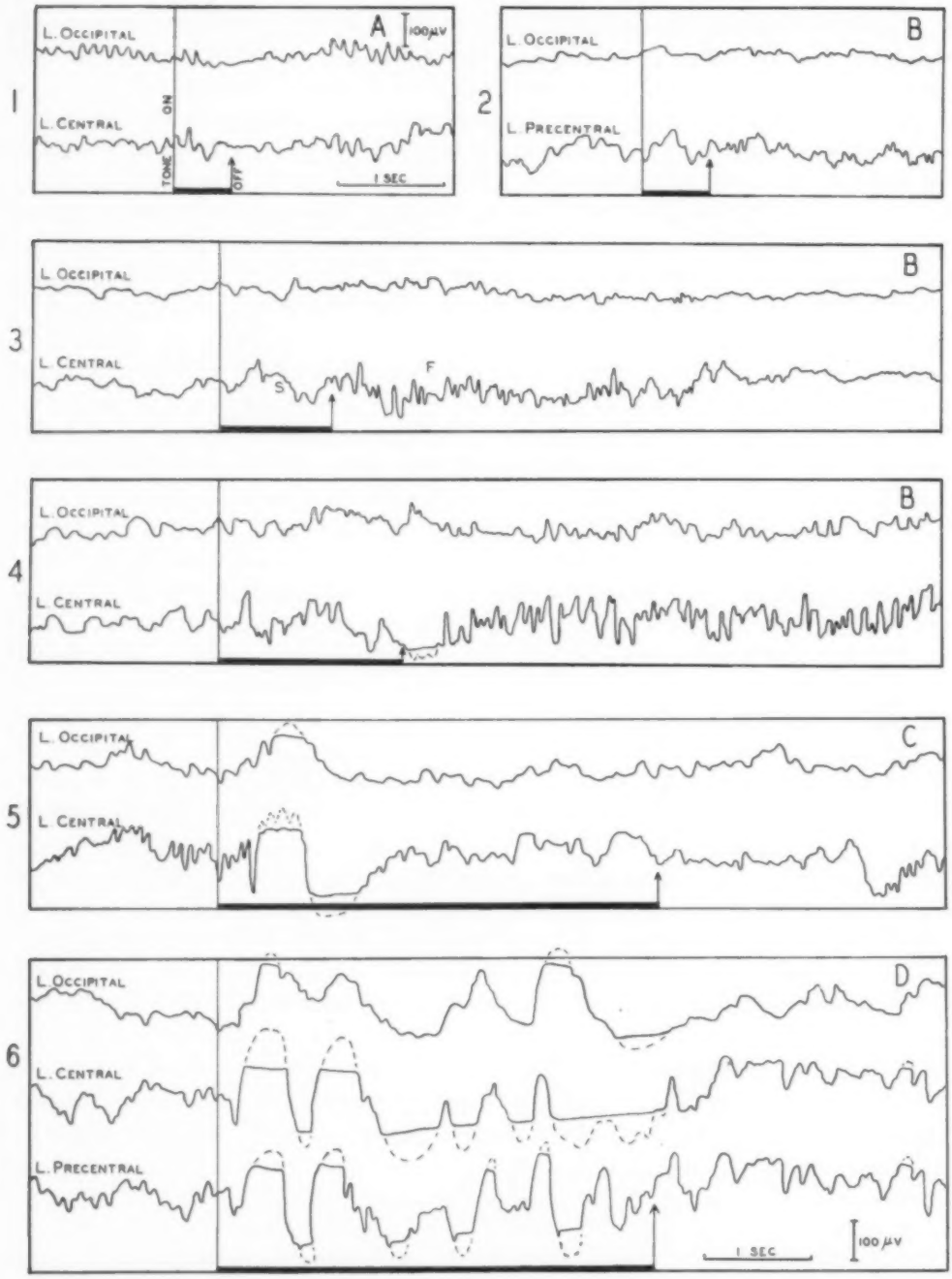

FIG. 3. Responses to tone of 500 cycles during the drowsy state and in sleep. Intensity of tone 70 db in 1, reduced to 55 for 2, 3, 4, 5, and 6. Noise-level about 25 db. Subject is a 14-yr.-old boy, who went to sleep for an afternoon nap. The stages of sleep are indicated by the lines in the upper right-hand corners. (See text for details.) Reference electrode on right mastoid region. Dotted lines indicate the probable approximate course of the potential-changes which are obscured by the current-limiting tube in the output stage.

record shows a strong alpha rhythm, which is almost continuously active (Fig. 3_1), and the alpha waves are unusually responsive to auditory signals. Perhaps the responsiveness is dependent upon the psychological conditions of the experiment, but this subject invariably showed a transient checking of his alpha rhythm whenever the tone was turned on, irrespective of whether he was instructed to turn it off or to pay no attention to it. As the subject became drowsy in this experiment his alpha rhythm became intermittent and returned abruptly when the subject turned off the tone. This reaction corresponds closely to the return from a "float" described in a previous paper (Davis, Davis, Loomis, Harvey, and Hobart, 1938).

The subject then passed into the low-voltage *B* stage of sleep. In this stage (Fig. 3_2) he continued to turn off the tone, although less promptly, but without any return of his alpha waves. The next modification (Fig. 3_3) was the appearance, following the tone, of fast waves at the central region. It should be clear from the central distribution and varied frequencies of these waves that they are not the usual alpha waves. They clearly represent the fast component of the typical *K*-complex. Fig. 3_3 also shows the first beginnings of the slow component, "*S*," during the stimulation. It is remarkable that the subject's EEG is now definitely in the "sleep" category, yet he continued to turn off the tone. From the beginning of the experiment the tone had been turned on automatically every half minute, and up to this point the subject had not once failed to turn it off. In Fig. 3_4 the record before stimulation is even more clearly a sleep record, with quite well-developed delta waves, and the *K*-complex following the stimulus is still better developed, but the subject still squeezed the bulb. The subject's reaction-time became progressively longer, as illustrated in Fig. $3_{1,2,3,4}$. The prolongation correlated well with the changes in electrical pattern, but only when the subject reached the *C* stage (Fig. 3_5), identified by the spontaneous train of 14-per-sec. waves appearing at the central region, did the subject fail to squeeze the bulb. The *K*-complex was then fully developed.

Fig. 3_6 shows the further development of the *K*-complex in the *D* stage of sleep. The fast-wave component has become less conspicuous. Its waves are slower and rounded and can scarcely be identified because of the high-voltage waves of the slow component, on which the fast waves are superimposed. In this *D* stage the slow (delta) activity becomes so rhythmic and so nearly continuous between stimuli that it is often difficult to determine whether there is or is not a response following a stimulus.

In the *E* stage, which was not reached in this experiment, there is no indication of any modification of the electrical record following even a very strong stimulus. It will be recalled that in the *E* stage the spontaneous delta activity is continuous at high voltage and at frequencies of 1 per sec. or less (cf. Fig. 6) and that in the *E* stage the trains of 14-per-sec. waves are absent.

The particular experiment illustrated in Fig. 3 is somewhat unusual in

the persistence of the motor reaction, apparently well into sleep. The progressive development of the *K*-complex is fairly typical, however, if we make allowance for individual characteristics of the *K*-complexes of various subjects. (For example, the prominent 6- to 8-per-sec. waves in Fig. 2 are characteristic of one particular subject and are quite well developed in two others.) A general rule seems to be perfectly clear. *The slow and the fast components of the K-complexes both resemble closely the delta waves and the fast waves which are characteristic of the spontaneous activity of the particular stage of sleep.* In fact, it now seems clear that most of the characteristics originally selected for the identification of the stages of sleep actually are the characteristics of the spontaneous *K*-waves, although the fast component of an evoked *K*-complex corresponds to the stage to which the subject is aroused rather than to the stage in which he was before being stimulated. The low-voltage *B* stage shows little delta activity, and the slow component of the *K*-complex is relatively small and fast (Fig. 3_3, 3_4, and Fig. 5). The fast-wave component is also relatively fast or may be represented by a more or less complete return of the waking pattern. The *C* stage of sleep has well-defined delta activity and prominent trains of 14-per-sec. waves. These trains obviously correspond closely to the fast component of the *K*-complex. The *D* stage is characterized by rhythmic delta waves which obviously resemble the rhythmic slow component of the *K*-complex.

Analysis of the K-complex

We have spoken throughout of the *K* "complex." We may obviously identify two main components—the slow-wave (delta) component ("*S*" in Fig. 2, 3, and 5) and the fast-wave component. Either the fast or the slow component may appear alone, or one may be well developed while the other is rudimentary. The two components may be separated experimentally by stimulation at brief intervals (Fig. 4). A second stimulus delivered within 4 or 5 sec. after a previous stimulus rarely evokes the slow component. If the slow component does appear, it is almost always reduced in size, faster in frequency, and often shows an unusually long latency. The fast-wave component, on the other hand, regularly appears following the second stimulus and merges with the fast-wave sequence of the first *K*-complex, as in Fig. 4.

If a tone is timed to fall 2 or 3 sec. after the major positive swing of a "spontaneous" *K*-complex, it usually fails to evoke a second slow component. The situation is the same whether the first *K*-complex is evoked by a known stimulus or is "spontaneous." It appears as if some part of the mechanism necessary for the generation of the slow component were refractory for several seconds after a previous response. The situation in regard to the fast component is quite different. Not only is its appearance not hindered by a previous *K*-complex, but it is often actually enhanced by it. Also a stimulus may fall in any relation to a "spontaneous" train of 14-per-sec. waves without hindering the appearance of either the slow or fast com-

ponent of the ensuing *K*-complex. The fast component alone does not leave the mechanism refractory for either the fast or the slow component. If there is a refractory period involved, it is so short as to be of quite another order of magnitude than that following the slow component.

If repeated stimuli are given at intervals of 3 or 4 sec. or less, the fast-wave activity tends to become faster and less regular, the waves sharper in contour but lower in voltage. The subject often stirs or awakens. Only if the subject is so deeply asleep that the fast waves of his *K*-response are

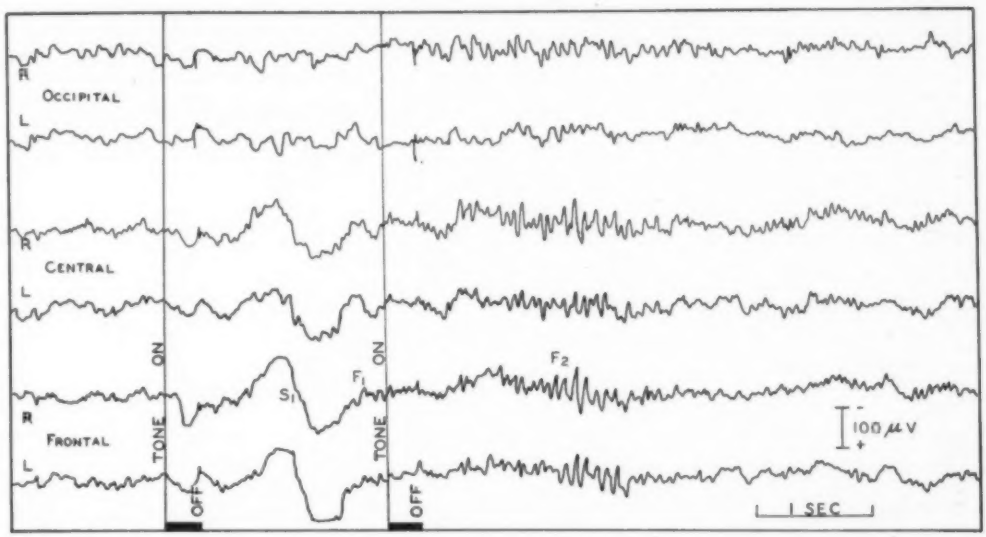

FIG. 4. Response to two brief tones at 2000 cycles at 45 db above threshold during *B-C* stage of sleep. Noise-level 35 to 40 db. Subject is a 41-year-old woman. The slow component, S_1, is of the frontal type. Note almost complete absence of slow components following the second tone, but the greatly increased fast component, F_2. Reference electrodes on right ear lobe for lines 1, 3, and 5; left ear lobe for 2, 4, and 6.

inconspicuous or are slower than 14 per sec. does such repetition of the stimulus fail to shift him to a lighter stage of sleep.

Varities of the slow-wave components

The *K*-complexes of many individuals in light sleep are both characteristic and reproducible. In Fig. 5 the fast-wave component is present, although poorly developed, and the slow rhythm is typical. The sequence begins with a small negative wave whose foot begins about 100 msec. after the stimulus. Successive waves are higher in voltage and longer in duration, so that no definite frequency can be assigned to them. The diagram in Fig. 5 is drawn from the average measurements of voltages and times of 13 *K*-complexes from the same individual. The 13 complexes were selected from 25 responses in approximately the same stage of sleep, because it was evident from actual superposition of the original records that they all resembled

one another quite closely. In a few cases, one or both of the first two small waves were absent, and in several the fourth and fifth waves were poorly developed, but all 13 clearly conformed to the same general pattern. The remaining 12 responses showed one, and usually more, of the diagrammatic waves at their appropriate intervals, but they were superimposed on and partially replaced by a somewhat slower sequence of rounder waves of approximately the same voltage (cf. Fig. 4, central and frontal, and Fig. 3_5). The pattern illustrated in Fig. 5 was always most prominent, *i.e.*, highest voltage, at the central region, while the slower, rounder waves of Fig. 4 were best developed in the frontal region. We therefore tentatively designate these two types of slow wave appearing in the *K*-complex as "central" and

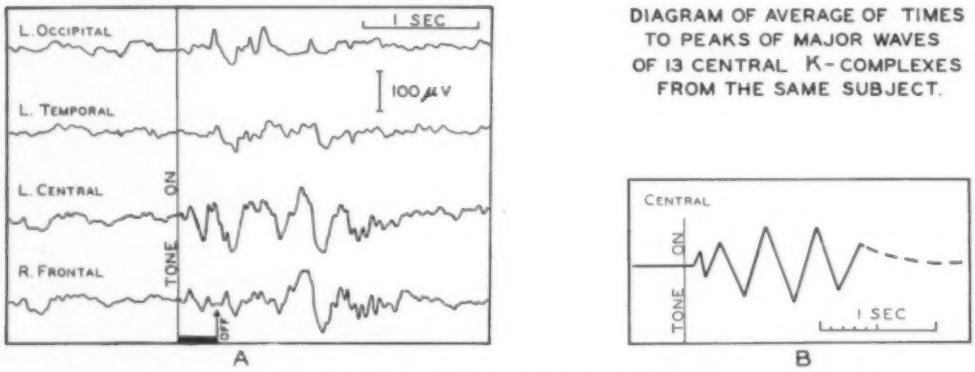

FIG. 5. *A*. Typical *K*-complex in response to a brief tone of 1000 cycles of 70 db above threshold from a 35-yr.-old man in the *B* stage of sleep. This response was selected as most typical of the "central" type of slow component. Reference electrodes on left mastoid region for three upper lines, on right mastoid for lowest.

B. A diagram of the composite of 13 "central" *K*-complexes from this subject.

"frontal," respectively. Some subjects gave a large proportion of pure "central" responses. Others tended to give more of the frontal variety, but more often the responses were mixed in character. In the "mixed" variety, both "central" and "frontal" waves appear both in the central and frontal areas, but the fast are more conspicuous in the record from the central region, and the slower, rounded waves are more prominent in the frontal area.

Intermittent auditory stimuli

The rather prominent 6- to 8-per-sec. "central" rhythm which appeared in some subjects (cf. esp. Fig. 2) and the apparent absence of refractory period for the fast-wave component led us to wonder whether the 6-per-sec. rhythm could be enhanced by stimulating with intermittent sounds (clicks or knocks) at a frequency of 6 per sec. It seemed possible that we might be able to "drive" the waves at frequencies determined by the frequency of stimulation, as Adrian and others have done with the occipital waves in the waking state (Adrian and Matthews, 1934; Loomis, Harvey, and Hobart,

1936). We were unable to produce a corresponding effect in sleep by auditory "flicker." Even rather loud knocks at frequencies near 6 per sec. produced no different response from what we regularly observed with steady tones. Knocking had, however, a much stronger tendency to awaken the sleeper, perhaps because of its intermittent character or because of its psychological associations.

Possible direct-current components of the K-complex

The time-course of the slowest waves and rhythms which appeared in the frontal region raised the question whether our amplifiers, with a time-constant of approximately 0.5 sec., were adequate to record all of the

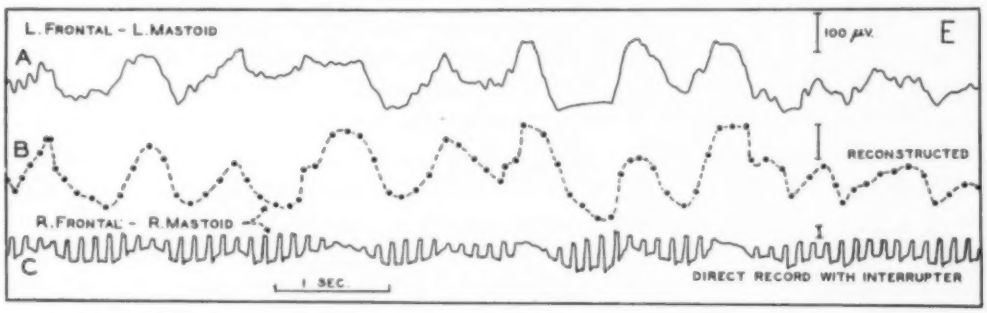

FIG. 6. Comparison of D.C. record of potential changes (line *B*) with a simultaneous record from the opposite side taken by the usual condenser-coupled amplifier. Large delta waves, *E* stage of sleep. *A* and *C* were recorded simultaneously. The input leads of line *C* were short-circuited automatically 8.5 times per sec. (see Davis, Davis, Loomis, Harvey, and Hobart, 1939). Line *B* is subsequent reconstruction from *C*. Distance of dots in *B* from a reference line (not shown) is proportional to the excursions in *C* on opening and closing of the short-circuit.

electrical response. It seemed possible that there might be a slow component,—a shift of the base-line,—as part of the *K*-complex which was not being revealed by our condenser-coupled amplifiers. We therefore employed the method of interrupting the input to one of our amplifiers (Davis, Davis, Loomis, Harvey, and Hobart, 1939) to determine whether such slow changes existed or not. Figure 6 shows how the potential appearing between the input electrodes when the short-circuiting switch is opened is revealed by the height of the square-topped "waves." The course of the potential-change is reconstructed in line *B* on a scale of sensitivity comparable with that of the usual record. Comparison between *A*, taken from the left side of the head, and line *B*, reconstructed from the record taken from the right side, shows that the usual method records with considerable fidelity the slow changes of voltage of the *E* stage of sleep.

Interrupted records taken during stimulation by tones could not follow the details of the *K*-complex, but showed conclusively that there was no slow shift of as much as 50 μV. in the electrical base-line associated with the *K*-complex.

Discussion

In the light of the foregoing experimental facts, the *K*-complex, or "disturbance pattern," of sleep appears as a multiple, diffuse, non-specific, delayed electric response of the brain to external sensory stimulation. It is clearly related to the on-effects seen in the waking state and also to the background of electrical activity characteristic of the stage of sleep in which it occurs. Auditory stimuli have so far proved most effective in evoking it. The effectiveness of auditory stimuli during sleep may be no accident if we consider the general biological function of hearing in the rôle of watchman constantly on guard to signal danger. In sleep the eyes are covered by the eyelids, and vision is in abeyance, but hearing remains active, ready to rouse the sleeper. The electric response is not confined to the auditory projection-area of the temporal region. On the contrary, it appears to be relatively weak over the temporal lobe, and best developed over sensorimotor and frontal areas. Its latency may be considerable, from 0.1 to a full second or even more.

The *K*-complex consists of at least two components,—the fast and the slow. In deep sleep the "fast" waves may be as slow as 10 or even 8 per sec. and resemble a smooth alpha rhythm. As the sleeper becomes more and more aroused, the "fast" waves become faster and less regular, with no clear 10-per-sec. rhythm. They appear to be more closely related to quick or beta waves than to the waking alpha waves (cf. Davis, Davis, Loomis, Harvey, and Hobart, 1938, p. 34). It seems reasonable to interpret the appearance of the fast component as an indication, and possibly even a measure, of the degree to which the subject has been aroused toward the waking state by the stimulus. Repeated stimuli at short intervals cause an increased (summated) fast component, and the subject is quite likely to awake. In deep sleep (stages *D* and *E*), the subject cannot easily be aroused, and the fast component of the *K*-complex is not at all prominent.

The slow component appears to be a definite response, often reproducible in considerable detail, clearly related to the waking on-effect. Its first waves correspond in latency, polarity, and duration to the waking on-effect. It seems reasonable to interpret the slow component as the waking on-effect modified and prolonged by sleep. Even before the subject goes to sleep, the on-effect becomes more prominent as the subject becomes drowsy. This increase in amplitude is broadly similar to the appearance in the animal brain of a delayed secondary discharge in stimulation of the sciatic nerve under deepening barbiturate anesthesia (Derbyshire, Rempel, Forbes, and Lambert, 1936; Forbes and Morison, 1939).

The slow component of the *K*-complex is rhythmic. If the same cells respond in the successive waves, we may be sure that their refractory period is shorter than 1 cycle of the rhythm. The slow-rhythm component as a whole, however, shows a refractory period to a second auditory stimulus, although the second stimulus may fall as much as 3 or 4 sec. after the original stimulus. This refractory period is much longer than 1 cycle of the rhythm. We

may infer that it is the avenue of approach to the cortex which is refractory, and not the responding elements themselves (cf. Forbes and Morison, 1939, for a theoretical discussion of the problem of the refractory state in a closely analogous situation). The refractory state may represent a true refractory period, like the long refractory period under dial anesthesia of the pathways of cutaneous sensitivity (Marshall, 1938), or possibly it is a secondary result of partial rousing of the subject, a shift in the general level of activity of the brain, which renders the brain less prone to this particular form of rhythmic activity. The effect might then be compared to the checking of the alpha rhythm by visual stimulation, attention, or an emotional state.

The slow component of the *K*-complex shows many similarities to the secondary discharge described by Forbes and Morison (1939) in the brain of the cat under barbiturate anesthesia. The resemblance is so close that it seems reasonable to attribute both phenomena to the same type of mechanism and to explain the quantitative differences as due to differences between the brains of the human being and of the cat, between sleep and barbiturate narcosis, and between sound and electrical stimulation of a peripheral nerve. Both phenomena are delayed cortical responses to sensory stimulation. Neither of them appears clearly in the waking state but they become prominent under conditions of depressed cortical activity (sleep and anesthesia) and both increase in size as the depression deepens. Both are generalized responses of the cortex. The latency is considerable in each case. Derbyshire, Rempel, Forbes, and Lambert (1936) state that the usual latency of their secondary discharge is 40 to 80 msec. We have not attempted to measure precisely the latency of the *K*-complex, because the first wave is often small and indefinite, but it is of the order of 100 msec. If ether anesthesia is given to an animal already deeply anesthetized by pentobarbital sodium (nembutal), the latency of the secondary discharge may be as great as 150 msec. (Forbes and Morison, 1939). The secondary discharge and the slow component of the *K*-complex both show a long refractory period in the sense that a response appears only after the first of a series of stimuli repeated at a rate of 4 per sec. The secondary discharge is reduced in amplitude unless the interval between stimuli is considerably more than 1 sec. The corresponding refractory period of the *K*-complex may last for as long as 4 or 5 sec. "Spontaneous" *K*-complexes, or secondary discharges, render the brain incapable of a second response just as effectively as do responses evoked by sensory stimulation. Forbes and Morison (1939) arrived at a similar conclusion concerning their secondary discharge. Bremer (1937) has emphasized the similarity of the electrical activity of the cat's brain in sleep to that under barbiturate anesthesia, in contrast to its activity under ether anesthesia. If our identification of our slow *K*-component with Forbes's secondary discharge is correct, it represents an important unification of observations on normal man with data from animal experimentation.

Forbes and Morison suggest that their secondary discharge may be analogous to the third (slowest and latest) component of the complex re-

sponse to stimulation described by Bishop and his collaborators (Bartley, O'Leary, and Bishop, 1937; Bishop and O'Leary, 1938). On the basis of the time relationships of the waves which he observed, Bishop suggested that his slowest waves represent activation of the mechanism which generates alpha waves in the waking state. Our evidence shows that the slow component of the *K*-complex is probably identical with Forbes's secondary discharge and also with the human waking "on-effect," but that it is clearly distinct from the human alpha waves. It appears therefore that one or another of the various comparisons is at fault. The situation is not disturbing, however, since some of the comparisons rest almost entirely upon similarity in time-relations and it is by no means a necessary conclusion that all electrical waves of similar frequencies represent activity of the same neural mechanism. For example, the "alpha" rhythm at 10 to 12 waves per sec. recorded by Grinker and Serota (1938) from the brains of cats under nembutal anesthesia is probably analogous to the human 14-per-sec. rhythm of sleep and to the "fast" component of the *K*-complex and not to the waking occipital 10-per-sec. alpha rhythm. Incidentally, Grinker and Serota report (*loc. cit.*, p. 575) a combination of slow and faster waves from the hypothalamus of the narcotized cat which corresponds closely to our cortical *K*-complex of sleep.

The appearance of *K*-complexes with two recognizable types of slow component, the "central" and the "frontal," suggest that two more or less distinct, but similar, neural systems may be set into action by the sensory stimulus. One system apparently tends to activate primarily the central, the other the frontal cortex, but sometimes the two systems seem to share the control of a given region, and we see the "mixed" type of response. The spread of "central" waves into the frontal region, and *vice versa*, is not a mere electrical artefact. Successive responses which are nearly identical in the precentral region often vary widely in their degree of spread into other regions. The cortical systems which generate the two types of waves apparently interdigitate to a considerable degree, and the extent of the response of each system varies from one test to another. Our experiments offer no indication whether a given neuron always participates in one, and only one, system or whether it may respond now in one and now in another pattern. The problem is exactly similar to the one raised by the alpha waves in the waking state in the precentral region. Sometimes they are 10-per-sec. waves in phase with similar waves at the occiput. At other times there are larger 8- or 9-per-sec. waves which are small or absent at the occiput (cf. Jasper and Andrews, 1938). Sometimes the 8-per-sec. precentral waves are coincident with low-voltage 10-per-sec. occipital waves, and the combination gives a confused record in which neither rhythm appears clearly, but in which both may be identified by careful inspection. Both in the waking and in the sleeping state we encounter a tendency of different regions of the cortex to react in rather similar ways, sometimes closely coördinated, yet at other times independently. When the reactions are independent, the indi-

vidual features characteristic of different regions may be recognized. The idea of independently reacting neural systems occupying the same cortical areas is not new. On-effects and secondary reactions have long been recognized (see Davis, 1939, for references), and in particular Bartley, O'Leary, and Bishop (1937) and Bishop and O'Leary (1938) have clearly distinguished two neural systems in the optic and also in the sensorimotor cortex of the cat (cf. also Adrian, 1936). The present evidence merely emphasizes once more the complexity of the organization and activity of the brain.

Summary

The electric response of the human brain to auditory stimuli during sleep, previously described and designated as the "*K*-complex," has been investigated in more detail. It is a multiple, diffuse, delayed, non-specific response to external sensory stimulation. Usually a fast component, consisting of a series of more or less regular waves at frequencies of 8 to 16 per sec., is superimposed on a series of slow (delta) waves (Figs. 2, 3, and 5), but either component may appear independently of the other. No shift of electrical base-line ("D.C. component") is associated with the *K*-complex (p. 501).

Light and electric shocks may elicit *K*-complexes, but less effectively than do sounds (p. 503). Typical *K*-complexes may appear "spontaneously" without assignable external cause.

The latency of the *K*-complex is usually of the order of 100 msec. and may be half a second or more. Both fast and slow components are more prominent in the central and frontal than in the occipital and temporal regions (Figs. 2 and 5). The patterns are simultaneous and broadly similar over all these regions, but characteristic differences between central and frontal types of the slow component are described (p. 507).

The fast component is often identical with the trains ("spindles") of 14-per-sec. waves which are characteristic of the *C* and *D* stages of sleep. The appearance of the fast component seems to represent a partial arousal of the sleeper. The slow component develops progressively from the waking "on-effect" (Fig. 1) and increases in amplitude and duration as the subject becomes drowsy and goes to sleep. Both components vary systematically with the stage of sleep (Fig. 3). The waves become slower as sleep deepens, and do not appear in the *E* stage of sleep. The characteristics of the spontaneous *K*-complexes are the chief criteria by which the *B*, *C*, and *D* stages were originally identified.

A sound within 3 or 4 sec. after a previous stimulus usually fails to evoke a second slow component. If it succeeds, the second response is delayed and reduced in amplitude. The fast component, on the contrary, appears full sized at all intervals of stimulation and merges with the fast component of the previous complex (Fig. 4).

Forbes and his collaborators have described a "secondary discharge" which appears in the cerebral cortex of the cat under barbiturate anesthesia

following electrical stimulation of the sciatic nerve. The slow component of the *K*-complex shows the same characteristics as, and is probably strictly analogous to, this secondary discharge (p. 512).

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CONDITIONING OF AFFERENT IMPULSES BY REFLEX DISCHARGES OVER THE DORSAL ROOTS

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A DESCRIPTION of the reflex discharge over the dorsal spinal roots, which takes place after stimulation of various sensory nerves, was presented in a previous paper. A general parallelism with the behavior of ventral root reflexes was pointed out, and from a review of the available histological evidence it was concluded that the cells of origin of the fibers carrying the reflexly excited impulses must be in the dorsal root ganglia. The latter view has now been fortified by the more recent evidence of Duncan and Crocker. Nothing was said in the previous paper, however, about what the outgoing impulses do in the periphery. It is now possible to state that the sensory mechanism can be conditioned by them. Experiments in which conditioning takes place will be set forth in the present paper, but before they are described it will be necessary to mention a number of points of contributory significance.

RESULTS

Effect of the temperature of the spinal cord

Recently Barron and Matthews (1939) have made the interesting observation that the size of the reflex discharge into the dorsal roots, evoked by a single afferent volley, is augmented if the spinal cord is cooled. From what is now known, following this observation, it appears likely that in our previous experiments, in which as many as 35 per cent of the alpha fibers were found to be accessible to reflex excitation, the temperature of the spinal cord may have fallen somewhat below normal. But, as will be shown later, this fact does not invalidate the significance of the experiments.

Because of the importance which the factor of temperature has acquired in arguments about dorsal root reflexes, a number of experiments were performed in order to gain additional evidence concerning the extent of the variation that temperature is able to produce.

The size of the reflex was first observed at normal body temperature. The rectal temperatures of 12 cats at rest in a warm room were measured and found to vary between 37.5 and 39.7° C., with an average of 38.5°. This average value was taken as the normal temperature, and the preparations were held as closely as possible to it by means of electric bulbs so arranged as not to radiate upon the back. Under these conditions, when the rectal temperature was normal, the spinal cord temperature, it was to be expected, would also be normal, as no operative procedures were carried out in the region of the spinal column. Decerebrated cats and cats under dial narcosis were examined. After exposure of the saphenous nerve, a pair of electrodes

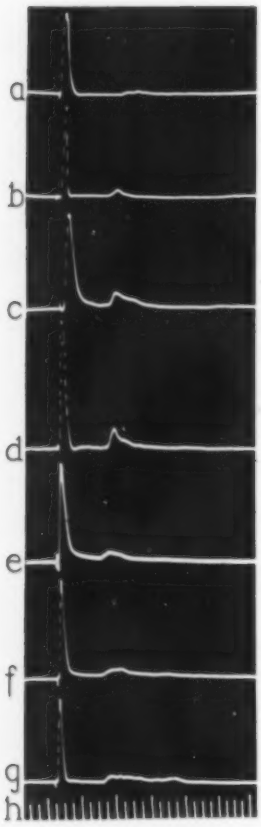

FIG. 1. Saphenous nerve to saphenous nerve reflex in cats at normal temperature (rectal). *a*, decerebrated cat at 39° C. *b*, decerebrated cat at 38.8°. *c*, cat under dial at 38°. *d*, cat under dial at 38°. *e*, decerebrated cat at 38.2°. *f*, the same preparation as *e*, after spinal section; temperature 40.7°. *g*, the same preparation as *e* and *f*, after application of cooling pads to the spine, following removal of the skin. *h*, 1 and 4 msec.

was applied to the end of the nerve in the way usually employed for obtaining a monophasic record of the impulses; and stimulating electrodes were applied central to the leads in order to evoke the afferent volley necessary for the production of a reflex. The records thus contain first, the potential produced by the afferent volley, then that of the reflex discharge. As the afferent volley contained all the alpha fibers, its potential was not subject to variations in size and, therefore, served as a convenient reference potential with which to compare the size of the reflex response.

Examples of records taken at normal temperature are shown in Fig. 1a–e. In every instance a well-marked reflex discharge is visible. Records a and b are taken from decerebrated cats after ample time had been allowed for excretion of the ether used during decerebration; c and d are from cats in dial narcosis. The reflex is relatively larger in the latter, despite the narcosis. Record e is again taken from a decerebrated preparation. This preparation was subsequently made spinal. Following the procedure, the temperature was found to have risen to 40.7° C. without producing much effect on the reflex (Fig. 1f). In order to compare the reflex obtained at the latter temperature with one at a colder temperature, the cord was cooled by denuding the lumbar vertebrae of muscles and applying pads soaked in cold Ringer's solution. After cooling, the reflex discharge increased, particularly in the portion succeeding the first burst of impulses (Fig. 1g). Similar effects were obtained in other preparations. Therefore, a series of experiments was set up in which variation of the temperature of the spinal cord could be accomplished with better control.

The temperature of the cord was measured with a thermocouple (constantan wire in a silver tube) applied through the back deeply into the seventh lumbar segment, on the side opposite to that on which the reflex was produced. Modification of the temperature was effected with the aid of copper tubes through which warmed or cooled water could be circulated. These tubes had a 3 mm. outside diameter and were threaded through the muscles of the back so as to lie on each side of the spinous processes from the lower thorax to the pelvis. This simple method had the advantage that it left the cord undisturbed, but it had the

disadvantage that all parts of the cord would not necessarily be at the same temperature. During cooling the dorsal part of the cord would tend to be colder than the ventral and *vice versa*. And the differential would be greater, the greater the deviation of the temperature of the water in the tubes from body temperature. As the dorsal horns of the cord were between the thermocouple and the cooling tube, it was therefore not to be expected that a series of observations at ascending temperatures would exactly match a series of observations at falling temperatures. The best conditions were those in which the water in the tubes was at a temperature close to that of the region about the thermopile. The temperature of the dorsum of the cord would then be between the observed limits.

Some variation in the effect of temperature was encountered from experiment to experiment; but the results in general were similar. They are fairly represented, as to the course and magnitude of the changes, by the experiment shown in Fig. 2. Record a shows the reflex with the cord temperature at 38.5° C. In size the reflex is comparable to reflexes shown for normal temperatures in Fig. 1. Lowering of the temperature to 30° C. produced a progressive increase in the height of the reflex (Fig. 2b, c, d), especially in the later portion of the discharge. At the normal temperature the reflex discharge, which continues until the end of the record, is in its later portion scarcely discernible at the amplication employed, while at 30° C. the discharge is well-marked throughout the record which is at the same amplification. A return to 38.5° C. restored the original dimensions (Fig. 2e). In the course of further warming, with the water in the tubes at 47° C., the reflex disappeared (Fig. 2f), although the thermocouple recorded only 40° C. This effect was undoubtedly due to the higher temperature in the dorsal horn, occasioned by the temperature gradient, and it disappeared when a stationary temperature of 40° was obtained with a tube temperature of 41° C. The picture at 40° C. then became hardly differentiable from normal (Fig. 2g).

FIG. 2. Effect of changes in the temperature of the spinal cord upon the saphenous nerve to saphenous nerve reflex. *a*, afferent volley and reflex at 38.5°. The spike size of afferent volley is valid for the records *a*–*g*. *b*, at 36.5°. *c*, at 34°. *d*, at 30°. *e*, rewarmed at 38.5°. *f*, at 40.1° in cord during the temperature increase. *g*, at 40°, 10 min. later at maintained temperature. *h*. 25 times higher amplification, only threshold fibers in the afferent volley (about 2 per cent of the alpha fibers) and the reflex at 38.5° of the spinal cord. *i*, as *h*, but at 31°. *k*, time 1 and 4 msec.

When small afferent volleys were used to produce the reflex, the temperature effect was much more striking. The afferent volley in Fig. 2h, repre-

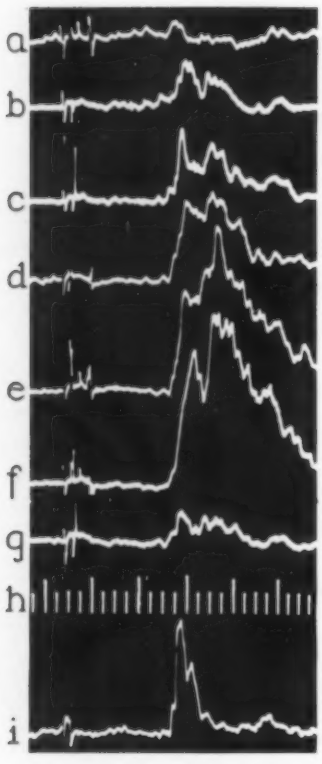

FIG. 3. Effect of changes in the temperature of the spinal cord on the dorsal root reflex in the saphenous nerve produced by a tap on the patellar tendon. *a*, at 38.5°. *b*, at 37.5°. *c*, at 36°. *d*, at 35°. *e*, at 33°. *f*, at 30°. *g*, at 38° rewarmed. *h*, time 1 and 4 msec. *i*, another preparation. Tap on the skin over tibia; reflex recorded on small side branch of saphenous nerve; rectal temperature 38.5°; time as in *h*.

senting about 2 per cent of the alpha fibers, produced a relatively larger reflex at a cord temperature of 38.5° C. than is produced by a larger volley. The area of the reflex potential is approximately equal to that of the afferent volley. But at 31° C. the potential during the first two milliseconds surpassed that of the afferent volley (Fig. 2i).

The effect of temperature was also striking when the reflex into the saphenous nerve was evoked by the afferent impulses arising from a tap on the patellar tendon. The tap was produced with the aid of a device prepared by modifying a small dynamic loud-speaker with a permanent magnet. The paper cone of the loud-speaker was removed from the moving coil and in its place a beveled rubber stopper was attached. In the primary winding of the regular transformer of the loud-speaker, a 1 μF condenser and a thyratron (type 884) were inserted. With this thyratron, which ordinarily served to synchronize electrical excitation with the sweep, the movement of the knee-jerk hammer (*i.e.*, the rubber stopper attached to the moving coil) could similarly be synchronized, as the thyratron controlled the discharge of the condenser (210 volts) through the coil. The secondary winding in the loud-speaker was grounded, in order to reduce the size of the electrical artifact produced in the record.

Figure 3 shows the records that were obtained. Early in the records there appears the electrical disturbance accompanying the tendon tap and this is followed by the potential of the reflex discharge into the saphenous nerve. Starting with a small reflex at a cord temperature of 38.5° C. (Fig. 3a), the reflex rapidly becomes larger as the cord is cooled (Fig. 3b–f). The increase is most rapid between normal and 33° C. Between 33° C. and 30° C. the rate of increase diminishes. On return to a cord temperature of 38° C. the reflex is again reduced (Fig. 3g).

Conditioning of afferent impulses

Afferent impulses were initiated in the saphenous nerve by a gentle tap on the skin of the leg. The tap was applied with the same device that was used for eliciting the knee-jerk, except that a flat piece of felt-covered wood,

5×2 cm. in size, was attached to the moving coil of the loud-speaker. The course of the free swing of the coil and its attachments, recorded by a method which translated the movement into potential change, is shown in Fig. 4a. The total duration of the swing including the recoil was 12 msec. When the skin was in contact with the surface of the stimulator during its movement the period was prolonged but slightly, and the principal effect of the contact was to reduce the amplitude of the swing. The strength of the tap was such that when it was applied to the back of the hand, it produced a sensation of mild pressure.

When the tap was applied to the skin over the tibia of the cat there resulted a discharge of impulses which lasted some 20 msec. and was characterized by a series of crests. The form of the discharge as recorded monophasically from the peripheral end of the cut saphenous nerve is shown in Fig. 4b. If the saphenous nerve fiber connections between the skin and the central nervous system were left intact and a branch of the saphenous nerve was prepared for leading, it was found that this physiologically excited discharge from the endings produced a reflex. Figure 3i shows a reflex produced in this manner in a cat, the rectal temperature of which was 38.5° C.

In order to ascertain whether interference with the afferent impulses is occasioned by the reflex discharge it is necessary to record the impulses from the saphenous nerve soon after the passage of the reflex discharge toward the periphery. From the nature of the experiment it is evident that the continuity of the nerve fibers between the skin and the spinal cord must be maintained. Diphasic leads from an exposed portion of the nerve are necessary and the situation is one which presents serious technical difficulties.

Because of the length of the discharges from the periphery diphasic recording is not suitable, for the evidence for the number of fibers involved is obscured by the fact that the second phase of the diphasic potential in some fibers tends to cancel out the first phase of the potential in others. Furthermore the difficulty, which is great enough with respect to the discharge in isolation, is greatly augmented when the latter is opposed to the reflex discharge. The reflex evoked by a single shock applied to the homolateral sciatic nerve lasts about 20 msec. When its impulses precede the afferent impulses, which have a similar duration, by a short enough interval to condition them, the two trains overlap in the nerve and the resulting potential picture is one in which it is almost impossible to determine with accuracy whether there is a deficit of impulses in the centrally directed stream as compared with the unconditioned stream.

A second source of confusion making difficult the interpretation of records of a nerve that has both peripheral and central connections with the body is in the interfering leads from the nerve at its points of junction of the freed portion with the body tissue. One electrode on the freed portion of the nerve may lead by way of the nerve and the body tissue the events occurring at the junction on the opposite side of the paired electrode. It is possible to reduce this source of error by freeing the nerve for a long distance, so that

the resistance of the nerve from the electrodes to the body may be made much higher than that in the interelectrode region. But even this precaution is not sufficient to avoid distortions which will interfere with measurements of small details in the potential. In order to eliminate completely unwanted leads by way of the body tissues, the leg was amputated just below the knee, leaving the saphenous nerve as the only connection between the leg and the rest of the body at the time the leads were made.

In order to preserve the excitability of the sensory endings in the amputated leg, the blood supply was maintained as long as possible. First the skin was severed. Then the muscles were divided and the fibula and tibia cut without disturbance of the major blood vessels. Only after the leg had been safely supported and mounted ready for stimulation of the skin by the tap from the loud-speaker system and the electrodes had been put in place on the nerve, were the blood vessels finally tied and cut. There then remained a period of 20 to 30 minutes during which the experimental manipulations could be carried out before asphyxiation made the sensory endings inexcitable.

The difficulty arising from the length of the discharges was met by cooling the nerve in the stretch from which the leads were made. The electrodes were inserted in a groove within a transparent bakelite block and provision was made for holding the block so that the free portion of the nerve would lie in the groove. When the nerve was in place, the groove was sealed with cotton wool soaked in liquid paraffin. Through channels about the groove within the bakelite block, water at any desired temperature could be pumped, and the temperature of the nerve thereby controlled. The cooled region of the nerve was 38 mm. long and the electrodes were 8 mm. from the ends. Therefore, the interpolar distance was 22 mm. From the nature of the arrangement it is apparent that impulses would enter the electrode block from the central end through fibers at body temperature, and from the peripheral end through fibers at room temperature (25° C.)

Cooling prolongs the refractory period. Beginning with the normal refractory period of about 0.5 msec. at normal body temperature, the refractory period increases as the temperature falls, slowly at first and then more rapidly, so that below 10° C. it comes to be between 20 and 30 msec. (Fig. 6m). This prolongation of the refractory period causes a simplification of the potential picture of the discharges. As the discharges are repetitive, the later impulses in the train fall within the refractory periods of the earlier ones and are blocked. How the blocking occurs among impulses coming from the sensory endings can be seen in parts c, d, and e of Fig. 4. In record e taken from the nerve cooled to 10° C. the picture is made up mostly of potentials belonging to the first component of the discharge. The latter now appears prolonged because of the increased temporal dispersion, and augmented in size because of the increase in area of the individual spikes.

That the impulses belonging to the reflex are also repetitive follows from their similar behavior in cooled nerve. Figure 5 shows records in the saphe-

nous nerve of reflexly excited impulses which have entered the cooling electrode from nerves at body temperature. A comparison of the records obtained at 28° C. with those obtained at 15° C. reveals a deficit in the later components in the cooler preparation, because of prolongation of the refractory period. A second consequence of the long refractory period is that the centrifugal discharge can block the centripetal discharge in its course

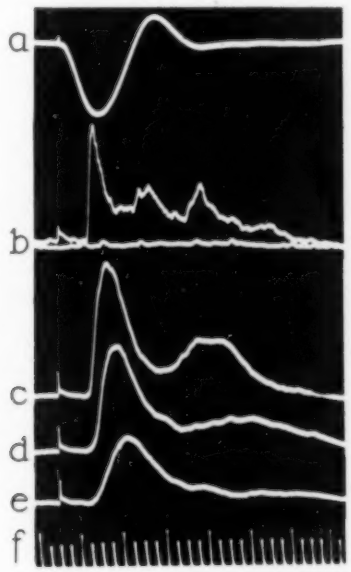

FIG. 4. *a*, course of the deflection of the loud-speaker coil used for production of mechanical stimuli. *b*, afferent impulses recorded monophasically from the peripheral end of the saphenous nerve (at 25°) as produced by a tap on the skin over the tibia. The lower line shows the level of spontaneous impulses and of noise. *c*, as *b*, except that the temperature within the electrode holder was lowered to 15°. *d*, as *b* and *c*, but at 12°, *e*, at 10°, *f*, time 1 and 4 msec.

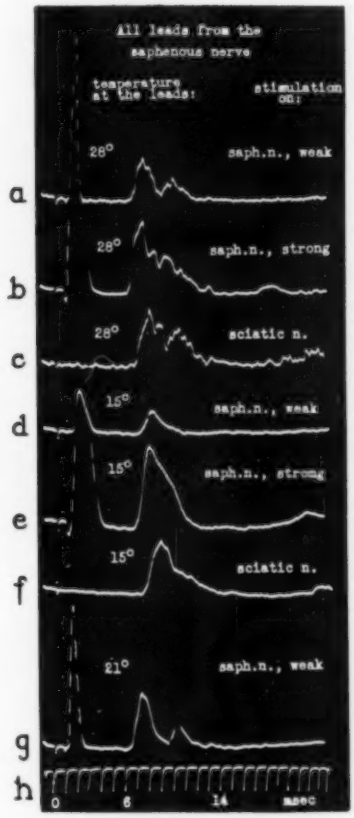

FIG. 5. Repetition of impulses in the dorsal root reflex discharge. The later parts of the reflex are blocked when the saphenous nerve inside the lead electrode is cooled to 15°.

through the nerve even when the one precedes the other by a considerable interval.

After these preliminary considerations it is now possible to follow the course of a conditioning experiment, such as that shown in Fig. 6. Records a and b were taken when the temperature within the electrode was 25° C. The first record, a, shows the afferent impulses. Two major sharp peaks downward complicated by secondary peaks are to be noted. At the arrow the

reflex evoked by these impulses appears. Its initiation is marked by two sharp peaks upward, because the impulses are running in the direction opposite to those which constitute the early part of the sequence. Record b

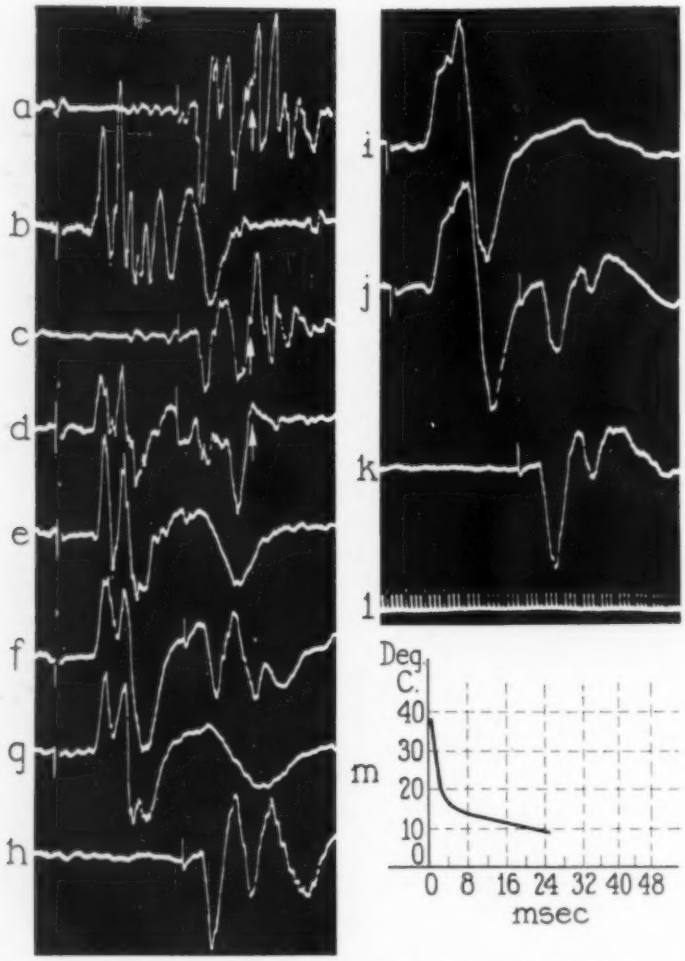

FIG. 6. Afferent discharges from sensory endings conditioned by dorsal root reflex discharges. Cat under dial. All leads diphasic from the uncut saphenous nerve. *a*, afferent impulses produced by tap on the skin over the tibia. *b*, reflex produced by a single electrical shock to the sciatic nerve. Records *a* and *b* taken at temperature of 25° within the perfused bakelite electrode with which the leads were made. *c*, tap response at 19°. *d*, sciatic nerve to saphenous nerve reflex, and the tap response as in *c*, at 19°. *e*, sciatic reflex alone at 19°. *f*, sciatic reflex and tap response at 13°. *g*, sciatic reflex alone at 13°. *h*, tap response alone at 13°. *i*, sciatic reflex at 10°. *j*, sciatic reflex and tap response at 10°. *k*, tap response at 10°. *l*, time 1 and 4 msec. *m*, total refractoriness of alpha fibers at different temperatures.

shows the reflex produced by a single shock applied to the homolateral sciatic nerve. The component at the end of the series, which is longer than the previous components, is the one travelling in delta fibers. It may be

identified in subsequent records at lower temperature, in which it appears delayed, and prolonged by increased dispersion.

When the discharges shown in records 6a and 6b were pitted one against the other, the record of the conditioning effect of the efferent discharge on the afferent discharge was too complex to be read and it is not shown. Uncertainty still attends the c, d, e series taken at 19°. But attention may be drawn to one feature of record d, *i.e.*, that the reflex produced by the afferent impulses from the skin has disappeared. The disappearance is attributable to inhibition of the reflex in the spinal cord, as a result of the conditioning effect of the activity set up there by the impulses that had previously arrived from the sciatic nerve.

However, in the f, g, h series taken at 13° C. a reading is possible. The potentials of both the discharges are now simplified and the delta component of the sciatic to saphenous reflex picture (Record g) is sufficiently delayed not to interfere with the measurement of the size of the first component of the sensory discharge. Record f shows that the first component is considerably reduced as compared with the control in Fig. h. Also careful comparison of an algebraic summation of g and h with the record of the potentials of the opposed discharges in f indicates that there is also a deficit in the second component of the afferent discharge.

On cooling the nerve in the electrode chamber to 10° C. the potentials were further simplified. The delta elevation in the sciatic-saphenous reflex (i) is now so delayed and flattened as not to interfere with either of the two components that make up the sensory discharge (k). It is obvious from record j that both components of the sensory discharge are reduced when the latter is conditioned. The reduction is well outside of the variation in the pictures of the sensory impulses obtained in a series of tests of the discharges in isolation.

FIG. 7. Afferent discharges from sensory endings conditioned by dorsal root reflex discharges. Cat under dial. *a*, *b*, and *c*, with nerve at 15°. *a*, sciatic to saphenous reflex. *b*, sensory discharge preceded by a sciatic to saphenous reflex. *c*, sensory discharge. *d*, *e*, and *f*, with nerve at 10°. *d*, reflex. *e*, sensory discharge preceded by reflex. *f*, sensory discharge. *g*, time 1 and 4 msec.

Conditioning of sensory impulses by an antecedent reflex discharge is also shown in Fig. 7. The records from the nerve at both 15° and 10° C. are favorable for the measurement of the deficits, as the delta elevation of the sciatic-saphaneous reflex is small at 15° C. and nearly invisible at 10° (Records a and d). Comparison of c with b shows that in b there is a deficit in both the components of the sensory discharge. The reflex recorded in c has been inhibited in b. When the nerve was cooled to 10° C., the record of the impulses from the sensory endings was reduced to a single component (f). This component was decreased by a preceding reflex discharge (e).

Discussion

All the experiments which were brought to a successful conclusion were in agreement in showing that afferent impulses arising from the skin as the result of mild mechanical stimulation, imitating the kind of stimulation that occurs hundreds of times a day in ordinary experience, are conditioned if they are preceded at an appropriate interval by a reflex discharge issuing from the spinal cord over the dorsal roots. A deficit in the quota of impulses in the discharge occurs, attributable either to failure of the impulses to be set up in the endings or to blockade in the fibers. In the several experiments the leading impulses in the reflex reached the periphery 4 to 13 msec. ahead of the slap and the first impulses were followed by later ones. From what is known of the effects of backfiring single volleys into the skin over sensory fibers it is certain that the number of impulses set up in the endings by the slap would be decreased at these intervals. In so far as impulses are set up in any of the fibers that had been occupied by the reflex, they would be blocked by the prolonged refractory period in the cooled region.

Because of the uncertainty about the number of impulses set up by the slap, the conditioning revealed in the experiments is susceptible of two interpretations. The first one depends upon the eventuality that the deficit of impulses observed arose from refractoriness or subnormality of the endings following upon participation of the fiber in the reflex or from prolonged refractoriness in the cooled segment of the nerve blocking impulses actually set up. In this event the observations would mean that sensory impulses and the reflex discharge are carried in the same fibers. The second interpretation demands the assumption of a special system of centrifugally conducting dorsal root fibers capable, when active, of conditioning sensory endings. Further work will be necessary before a decision can be made between the two possibilities. The only conclusion permissible at present is that reflexly evoked impulses have an intimate relationship to the sensory mechanism.

The experiments were carried out before the great importance of the temperature of the spinal cord as a determinant of the size of the reflex was realized; and as special precautions to maintain the temperature of the cord were not taken, the temperatures in all probability dropped somewhat below normal. This drop would augment the reflex and therefore

facilitate conditioning, but the significance of the fact that conditioning occurs would not be obscured thereby. However, no conclusion is possible about the extent to which central conditioning of peripheral endings enters into the physiology of sensory mechanisms.

Summary

When the action potential of the sensory impulses conducted through the saphenous nerve of the cat following a gentle tap on the skin is recorded from the side of the nerve, it is found that a deficit in the total number of impulses occurs if the train is immediately preceded by a reflex discharge through the nerve from the spinal cord.

The experiments conducted in the course of the present investigation permit the mention of a number of characteristics of the reflex discharge by way of the dorsal roots, not included in the previous report:

a. The reflex discharge into the individual fibers is repetitive.

b. The reflex can be evoked by physiologically selected afferent impulses, as those set up by a tap on the skin or to the patellar tendon.

c. As shown by Barron and Matthews, the size of the reflex is greatly augmented if the cord is cooled. However, well defined reflexes are regularly present when the cord is at a normal temperature.

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SPINAL PATHWAYS FOR NICTITATING MEMBRANE REFLEXES IN THE CAT

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THE NICTITATING membrane (n.m.) of the cat has, in recent years, taken a place beside the cardiovascular system as a convenient indicator in the elucidation of autonomic function. In addition to the n.m.'s use in analysis of humoral, synaptic and myoneural junctional phenomena, its reflex activity has been studied from several standpoints. Rosenblueth and Bard (1932) have reviewed the structure and innervation of the n.m. The characteristic forms of its reflex responses to various types of peripheral afferent stimuli have been described in detail by Rosenblueth and Schwartz (1935), with particular reference to excitatory rebound, after-discharge and absence of parallel sign with concomitant vasomotor reflexes. The peripheral afferent nerves giving these responses have been investigated by Acheson, Rosenblueth and Partington (1936). Morison and Rioch (1937) studied the influence of forebrain and midbrain mechanisms on the reflex responses of the n.m. under urethane anaesthesia by methods of cortical and subcortical stimulation and extirpation. They concluded that at least three supraspinal areas contributed to the excitatory components and two or more to the inhibitory components of the n.m. reflexes. No responses were obtained following transection of the brain stem caudal to the midbrain. They further confirmed the lack of parallelism in sign and form between vasomotor and n.m. responses to identical stimuli. Watkins (1938) has extended this observation in taking simultaneous records of B. P. and n.m. changes during distention of hollow viscera.

In connection with these studies the spinal pathways of n.m. reflexes become of interest, and particularly so since Ranson and his coworkers (Ranson and von Hess, 1915; Ranson, 1916; Ranson and Billingsley, 1916a and b) showed that the spinal paths of the depressor and pressor reflexes, respiratory reflexes and "pain" were not localized in the same parts of the spinal cord.

METHODS

Adult cats were used throughout. In chronic preparations cord lesions were made with fine pointed scissors following aseptic laminectomy; dural slits were left open and wounds were closed with silk in layers. No wound infections were encountered. All experiments were performed under urethane anaesthesia (1.3–1.8 g. per kg.) injected intravenously after preliminary brief etherization. N.m. movements were recorded isotonically by a light, counterbalanced heart lever with a magnification of 13 to 18 times. In some experiments respiration was recorded by a tambour attached to a side arm tube of the tracheal cannula, and blood pressure tracings were made by a mercury manometer connected with a carotid cannula. Both sciatic nerves and a bundle of brachial nerves, prepared after the method of Ranson to equal roughly the cross section area of a single sciatic nerve, were used as afferents. Thus in each experiment, n.m. tracings were obtained from levels both caudal and rostral to spinal segments wherein lesions were made. Buried shielded electrodes were

applied to nerves following peripheral crushing. In a few instances the hind legs were denervated; this procedure was found not to affect results significantly and therefore was not followed routinely. Stimuli were induction shocks at tetanizing frequency from a Harvard inductorium with 3 v. in the primary circuit.

In acute experiments cord sections were made immediately after laminectomy. Kymograph tracings were taken immediately before and up to 4 hr. after sections; the animals were kept under light anaesthesia throughout by small supplementary intravenous urethane injections. In two experiments the carotid sinuses were denervated and the vagi were cut bilaterally with consequent augmentation of those n.m. responses still present in the preparation but no restoration of those responses already abolished by cord lesions above the level of stimulation. Hence these procedures were routinely omitted. The level of the lesion was determined by autopsy following each experiment, and the extent of the lesion was checked by either freehand or serial sections of involved cord segments fixed in formalin.

Results

1. *Total section:* In three cats, of which two were acute and one a six-day post-operative preparation, complete cord section at L3, T9, and T10 respectively abolished n.m. response on sciatic stimulation; brachial control readings showed responses within normal limits.

2. *Posterior column section:* The posterior columns were cut in five cats; in three of these the lesion was limited to the posterior columns alone, and

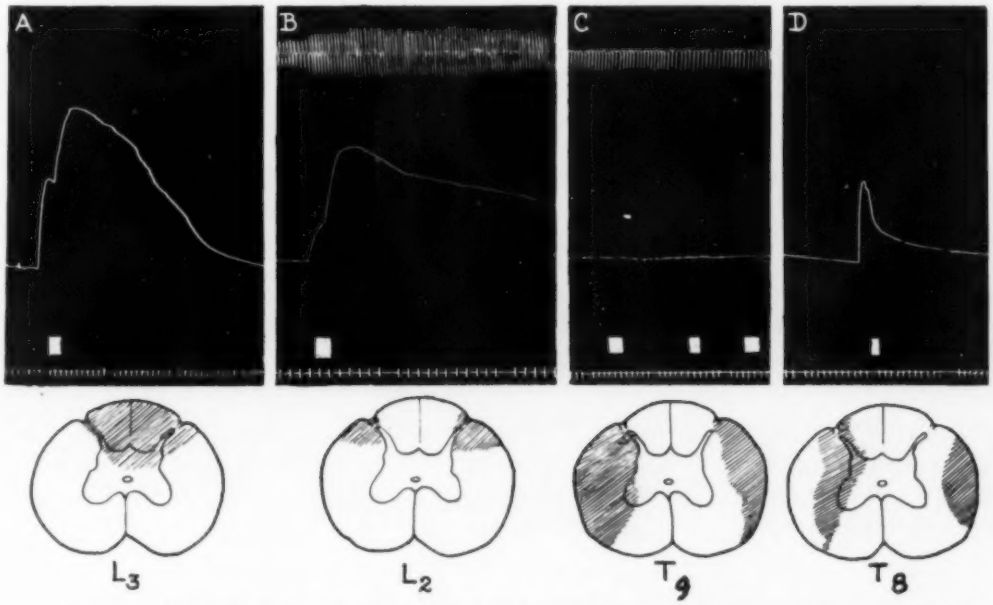

Fig. 1. Nictitating membrane responses to sciatic stimulation in cats with bilateral lesions of the spinal cord. Records, reading from below upward, show: Time, in 5-second intervals; Signal marker of stimulation; Nictitating membrane record; (in B and C) Respiration record. The diagrams below each record are representations of the extent of the lesion as determined by serial sections.

A. Section of posterior funiculi at L3. Left sciatic stimulated, 7 cm. coil distance.

B. Lissauer's tracts at L2. Left sciatic stimulated, 6 cm. coil distance.

C. Lateral columns, rt. complete, lt. outer half, at T9. Left sciatic stimulated, 12, 10, and 4 cm. coil distance respectively (cat 39, Table 1).

D. Outer half rt. and inner half lt. lateral column at L3. Left sciatic stimulated, 4 cm. coil distance. (cat 4a, Table 1).

placed at C6, T8, and L3 respectively; in the fourth and fifth the posterior columns were cut in the course of posterior hemisection at T6 and L3. In all cats autopsies showed lesions to be complete. In the instances of T8 and L3 lesions, the n.m. responses from stimulation both above and below were

Table 1. Nictitating membrane responses evoked by sciatic and brachial stimulation after bilateral lesions of the lateral columns of the spinal cord in the lower thoracic region.

Cat number	Level and lesion	Sciatic stimulation			Brachial stimulation			Time post-op.
		coil distance	rise	re-bound	coil distance	rise	re-bound	
9a	T8, lat. cols. complete. Small hemorrhage in ant. cols.	2 cm.	0	0	5 cm.	++++	++	1½ hrs.
39	T9, lat. cols. rt. complete; lt. outer half only*	4 cm.	0	0	4 cm.	+++	+	8 days
4a	T8, lat. cols. rt. outer half cut; left inner half cut, outer half intact.**	4 cm.	++	0	*no record*			2 hrs.
200	L3, lat. cols. complete	6 cm.	0	0	6 cm.	++++	+	5 days
7a	L2, lat. and ant. cols. complete	1 cm.	0	0	6 cm.	++++	++	1 hr.
46	L3, lat. cols. rt. and lt. incomplete	6 cm.	++++	0	6 cm.	++++	+	7 days.

* Figure 1, C. ** Figure 1, D.

normal in initial amplitude and in the extent of the excitatory rebound (Fig. 1A). The n.m. response following posterior column section at C6 consisted entirely in excitatory rebound for the first four hours, but thereafter resumed its preoperative contour.

3. *Lissauer's tract:* The apices of both posterior horns and the adjoining tracts of Lissauer were cut bilaterally in five cats. In two cases the lesions were limited to these areas alone and placed at the same level, T9 and L3 respectively. In a third Lissauer's tracts were sectioned on one side at L2 and on the opposite side at T8. In the two remaining cats the tracts were cut in the course of the posterior hemisections at T6 and L3 mentioned in

the preceding paragraph. Autopsies showed complete lesions. In all cats strong n.m. contraction with normal rebound followed sciatic stimulation (Fig. 1B).

4. *Lateral columns:* The lateral column was cut on both sides in the same segment in six cats, and the n.m. reflexes were tested at intervals varying between one hour and eight days post-operatively. The results are

Table 2. Nictitating membrane responses evoked by sciatic and brachial stimulation after hemisection of the spinal cord in the upper lumbar and contralaterally in the mid thoracic regions.

Cat number	Level and lesions	Sciatic stimulation			Brachial stimulation			Time post-op.
		coil distance and side	rise	rebound	coil distance	rise	rebound	
202	L3 lt. hemisec. T8 rt. hemisec.	Rt. 6 cm. Lt. 6 cm.	0 0	0 0	6 cm.	+++	+	left hemi. six days before exp. rt. hemi. during exp.
203	L3 lt. hemisec. T6 rt. hemisec.	Rt. 3 cm. Lt. 3 cm.	0 0	0 0	6 cm.	+++	++	2 hrs.
14	L3 lt. hemisec. T7 rt. hemisec.	Lt. 4 cm. Rt. 4 cm.	0 0	0 0	6 cm.	+++	++	2 hrs.
15	L2 rt. lat. col. only T8 lt. hemisection	Lt. 6 cm. Rt. 5 cm.	0 0	0 0	6 cm.	+++	+	6 days.

summarized in Table 1, and show that when the lesions of the lateral columns were complete there was no response of the n.m. to sciatic stimulation, though normal reflexes were elicited from the brachial nerves.

5. *Crossing of pathways:* Single hemisections were made in three cats at T10, T12, and L3 respectively. N.m. tracings were made one to two hours post-operatively and showed responses normal and equal in extent and rebound on stimulation of either sciatic. To determine the lower limit of crossing, the cord was exposed from L2 down to the cauda equina in one cat. Successive hemisections on the left were made in each segment throughout the lumbar cord; after each hemisection n.m. tracings were taken from each sciatic. After the second hemisection (L3) rebound disappeared on both sides, but responses equal in extent and approximately one-third that of the pre-operative reflexes could be obtained from either sciatic. A single hemisection placed at L1 on the right, *i.e.*, one segment above and

contralateral to the original hemisection, abolished sciatic n.m. responses; brachial stimulation was still effective, but the responses were about one-half as strong as in the pre-operative control period and there was no rebound. Autopsy showed all lesions to be either complete hemisections or to be slightly more than complete.

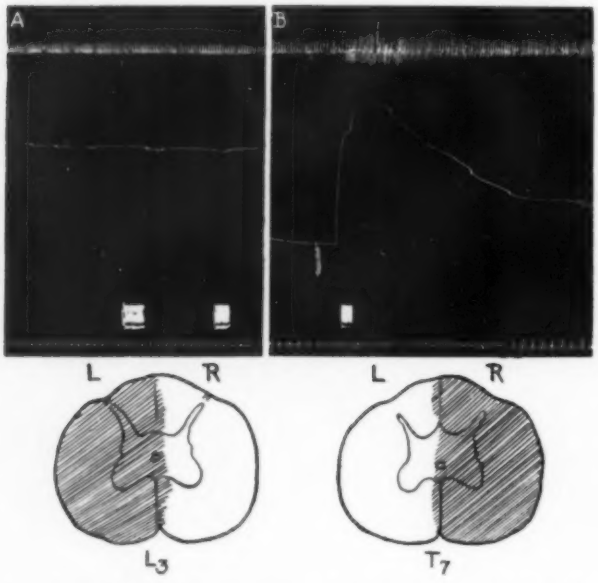

FIG. 2. Nictitating membrane responses to sciatic and brachial stimulation in cats with hemisection of the spinal cord in the upper lumbar and contralateral hemisection in the mid thoracic region. Records as in Fig. 1. Diagrams show extent of lesion at L3 and T7 respectively. (Cat 14, Table 2).

A. Sciatic stimulation, left and right respectively, 4 cm. coil distance.

B. Brachial stimulation, 6 cm. coil distance.

To determine whether crossing occurs in the upper lumbar and lower thoracic cord, above the level of entry of the sciatic fibers, contralateral hemisections at upper and lower levels of this region were studied in four cats. The results are summarized in Table 2, and show that one hemisection of the spinal cord at L3 or L2 and a second, contralateral, hemisection at T6 to T8 abolish all n.m. responses to stimulation of either sciatic, though the responses to brachial stimulation remain essentially normal (Fig. 2).

In five cats hemisections were made between C5 and C6, together with contralateral lower hemisections, and records were taken from both membranes. All five cats were operated four or more days before the final experiments. The results were inconstant. In two cases essentially normal reflexes were obtained in both membranes on sciatic stimulation. In three cases the membrane ipsilateral to the cervical hemisection responded normally or without rebound, whereas the contralateral n.m. responded poorly or not at all. In all cases the responses to brachial stimulation were normal and equal.

DISCUSSION

Using the sciatics as afferents, Ranson and coworkers (Ranson and von Hess, 1915; Ranson, 1916; Ranson and Billingsley, 1916a and b) have mapped the lower thoracic and upper lumbar spinal pathways for the vasomotor autonomic reflexes in the cat. They found the pressor afferent pathway from a given sciatic to lie bilaterally in Lissauer's tract, with conduction somewhat better ipsilaterally; the depressor pathway bilaterally in

the anterior part of the lateral columns, with conduction somewhat better contralaterally. Reflex acceleration of respiration was reduced but not abolished by bilateral section of either or both of these regions, or by posterior hemisection. These workers, however, were unable to demonstrate any hypalgesia to "conscious pain," as evidenced by struggle and cry in unanaesthetized chronic preparations, with either posterior hemisection, lateral hemisection, bilateral sections of lateral columns, or two hemisections placed on opposite sides of the cord a few segments apart. The degree of crossing of the vasomotor reflexes above the level of sciatic entry was not specifically investigated.

Harper and McSwiney (1937) have found that afferent pathways for reflex pupillary dilatation on stimulation of either hypogastrics or intercostals lie both ipsilaterally and contralaterally in the lateral columns, with all crossing in the segment of entry. In the experiments reported here it has been demonstrated that normal n.m. reflexes are evoked by stimulation of either sciatic after section of the posterior funiculi (p. 528) or after bilateral destruction of the apices of the posterior horns together with Lissauer's tracts (p. 528). Complete section of the lateral columns on both sides abolished the responses (p. 529). Moreover, in cat 4a (Table 1, and Fig. 1D) the superficial half of one lateral column was preserved and the n.m. responses were present, though reduced, from both sciatics. It may be concluded that the afferent pathways in the spinal cord lie in the lateral columns, the fibers being probably diffusely distributed in the superficial parts of these columns. Since equal responses were obtained from either sciatic following hemisection of the cord (p. 529), it would appear that conduction is equal in both crossed and uncrossed pathways. The abolition of responses by two hemisections, one at L2 or L3 and the other contralaterally at T4 to T8 (p. 529) indicates further that the crossing occurs within one or two segments from the level of entry and that there is no further crossing in the upper lumbar or lower thoracic portions of the cord.

In these respects the lumbar and lower thoracic spinal course of the afferent path for the n.m. reflexes is similar to that described by Harper and McSwiney (1937) for the pupillodilator reflexes. It differs from the afferent path for the vasodepressor reflexes as described by Ranson *et al.* in two respects: (i), since ipsi- and contralateral conduction are approximately equal by the methods used; and (ii), the pathway is apparently diffusely distributed in the superficial half of the lateral columns.

The experiments on combined, contralateral hemisections at L3 and C5 or C6 (p. 530) indicate that the spino-spinal afferent paths from both above and below the level of motor outflow to the n.m. (Langley, 1895) cross below C6 and above T8 respectively. It seems probable that this crossing is at the level of the motor outflow itself. Since Morison and Rioch (1937) found that the rebound phenomenon in the reflexes was dependent on the integrity of certain forebrain mechanisms, and that no reflexes were obtained following transections below the medulla, it would seem likely that the

reflexes dealt with here are spinal reflexes, facilitated by forebrain mechanisms the efferents of which cross below the level of C6. The other possible explanation for the phenomena under consideration would be that the reflex arc includes forebrain mechanisms and that the afferents cross to a large extent between T6 and C6. This seems less probable.

Langworthy and Richter (1930) evoked strong skin galvanic responses by stimulation of the posterior column nuclei, and Cannon and Rosenblueth (personal communication) obtained large n.m. responses by stimulation of the central ends of the cut, isolated posterior funiculi in the upper cervical region. In view of these observations it is of interest that section of the posterior funiculi in the thoracic or cervical levels was without effect on the n.m. reflexes in either form or extent in the present experiments.

Summary and Conclusions

The afferent pathways through the spinal cord for reflexes of the nictitating membrane (n.m.) in cats were studied by recording the n.m. responses to sciatic and brachial stimulation under urethane anaesthesia after localized lesions of the spinal cord.

It is concluded that the pathways lie in the lateral columns of the cord and are approximately equally crossed and uncrossed. Crossing takes place at the level of entry of the sciatic posterior roots and no further crossing can be demonstrated between this and the lower limit of the level of the sympathetic outflow to the n.m. Crossing again occurs at the n.m. center in the thoracic cord both for spino-spinal afferents from the brachial and sciatic nerves and probably for forebrain efferent mechanisms.

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VAGAL INHIBITION OF INSPIRATION, AND ACCOMPANYING CHANGES OF RESPIRATORY RHYTHM

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THE RESPIRATORY center, during each inspiration, normally receives impulses transmitted over the vagi from stretch receptors in the lungs. A state of central inhibition is thus built up, and eventually it cuts short the motor discharge. The actual mechanism of this effect is unknown. Both temporal and spatial summation of inhibitory impulses must be involved; for as the lungs are inflated there is a progressive recruitment in the number of active stretch receptors, together with an increase in the frequency of impulses from each unit (Adrian, 1933; Partridge, 1935). Inhibition might conceivably neutralize the driving force of inspiration, as it has been considered to inactivate quantitatively the central excitatory state (*c. e. s.*) in spinal reflexes (Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932). Inspiration might then stop because the driving force is exhausted, the depletion resulting partly from neutralization, partly from expenditure in cellular activity. On the other hand, there may be no direct neutralization; inspiration ceasing because inhibition, when built up to a critical level, renders the center refractory to *c. e. s.* In the latter case the center, as it discharges, might still become increasingly susceptible to inhibition. It is possible that the resistance to inhibition might also be found to vary with alterations in the tension of CO_2 or of O_2.

It would be difficult, under natural conditions, to test these several hypotheses. The normal inflation reflex may, however, be eliminated by sectioning both vagi. Inspirations can then be regularly cut short by properly timed central stimulation of one vagus (Hillenbrand and Boyd, 1936). Under these conditions it is possible to observe the response of the center to groups of vagal volleys, the number and temporal distribution being controlled.

In a preliminary report Boyd and Hillenbrand (1937) described the effects of stimulating one vagus, during inspiration, for varying short periods of time. A single volley of impulses was never sufficient to inhibit. A definite "inhibitory threshold" could be found and expressed as the minimum number of volleys, or minimum time of stimulation required (frequency and shock voltage remaining constant). A threshold series of volleys would cut short inspiration promptly and completely, whereas a subthreshold series failed to reduce the amplitude at all. Graded reduction of inspiratory depth could be effected by varying the time at which stimulation began, but not by grading the number of inhibitory volleys. Apparently, therefore, inhibition does not quantitatively inactivate the driving force of inspiration. It

acts in an all-or-none manner, and the stopping of inspiration at any given point depends upon the intensity of inhibition existing at the moment.

The methods used in the present study make it possible for stimulation to be repeated at the same stage in successive inspirations. The stage at which it begins, the frequency, the voltage, and the duration of the period of stimulation, can all be controlled independently. We have made observations upon (a) certain effects appearing with a near-threshold series of volleys; (b) variations of the inhibitory threshold at different times during the course of inspiration; (c) the effects of increasing the respiratory dead space; and (d) the acceleration of rhythm which accompanies a reduction of amplitude.

It is recognized that when the vagus trunk is stimulated an inhibitory effect upon respiration may be due in part to afferent fibers not of pulmonary origin (*e.g.*, from pressure receptors in the aorta). The electrodes were applied from 4 to 8 cm. below the origin of the superior laryngeal branch. A further complication is that fibers with an excitatory action upon the center may be stimulated simultaneously with the inhibitory group. Apparently the vagus trunk does contain fibers mediating pain sensations (Heinbecker and O'Leary, 1932); and the responses of lightly anesthetized animals to central stimulation of the vagus are somewhat variable. Tetanic stimulation for only 0.5 sec. may leave reflex after-effects of increased rate and amplitude persisting for several cycles. This is true particularly if strong stimuli are used. With adequate anesthesia, however, and with care to avoid unnecessarily strong stimuli, the effects of such brief stimulation are quite constant and are limited to a single cycle. The question of mixed excitatory and inhibitory effects will be again considered in section (d) below.

METHODS

Dogs were used, anesthetized by means of sodium barbital given intraperitoneally. The usual dose was 0.3 g. per kg., but in some instances this failed to induce a complete anesthesia and supplementary doses were given by slow intravenous injection. A tracheal cannula was inserted. Both vagi were sectioned in the low cervical region, and the central stump of one, ordinarily the left, used for stimulation. The electrodes, and the arrangement for recording respiratory changes of intrapleural pressure, were similar to those employed by Hillenbrand and Boyd (1936).

The stimulating circuit (Fig. 1) is based upon that described by Schmitt and Schmitt (1932). Brief induced shocks are set up by rhythmical discharges of a condenser (C_1 to C_3) through a coreless coil (T_1). We ordinarily used a single condenser of 0.02 μF., varying the frequency of discharge by adjusting the charging resistance R_1, and the stimulus strength by varying the shunt resistance R_6. Frequencies employed ranged from 80 to 140 per sec. The voltage necessarily was varied for different nerves, but was just sufficient in each instance to maintain, with continuous tetanization, a reflex apnea for a time equivalent to several cycles. Voltage and frequency are constant throughout each of the graphic records shown.

We modified the circuit of Schmitt and Schmitt by placing two RCA 885 tubes (A and B of Fig. 1) in parallel. The condenser discharges can be led through either tube by adjusting the grid potential of A, that of B being fixed. Stimulation takes place only when the discharge is through B, that tube being in series with the induction coil T_1.

With the switches K_5 and K_6 both open, the grid potential of A is low enough to divert all discharges through it, and the stimulating circuit is inactive. Closure of K_5 puts a fixed high potential on grid A, resulting in continuous tetanization until K_5 is opened again. Or, K_5 being left open, stimulation may be applied for any desired period, from a single shock

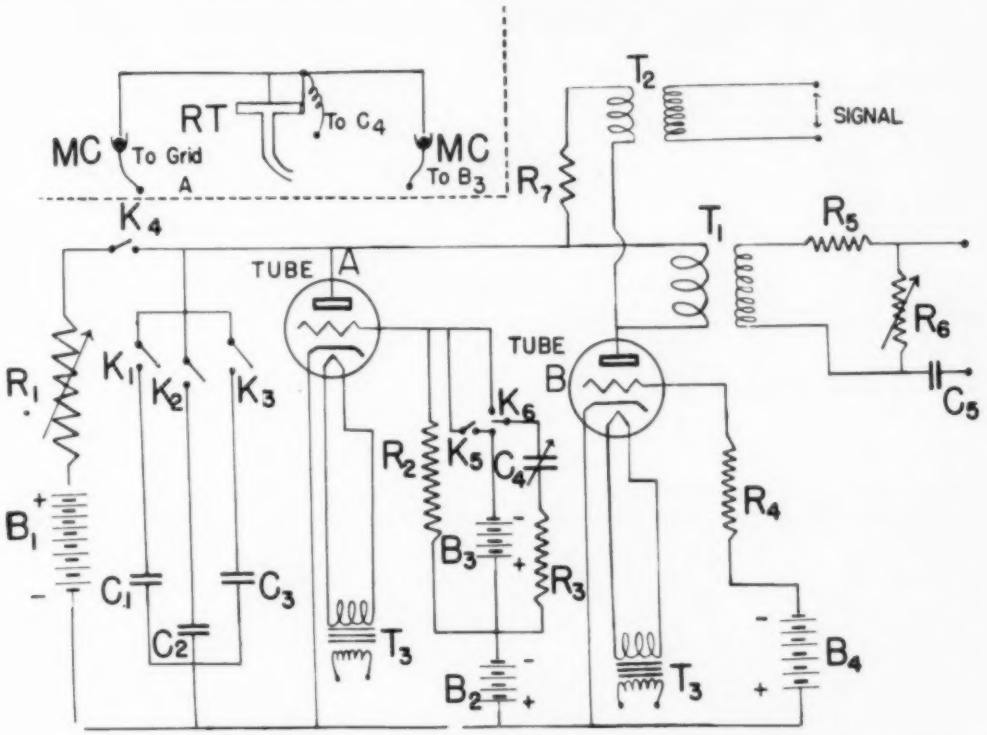


FIG. 1. Details of stimulator. A and B, grid-controlled, gaseous-discharge tubes (RCA 885). B_1, B_2, B_3, B_4, batteries of 157.5, 9.0, 13.5 and 13.5 V., respectively. R_1, resistance of 1 M Ω, variable in steps of 10,000 Ω. R_2, R_3, R_4, R_5, R_7, fixed resistances of 200,000, 100,000, 20,000, 1000, and 500 Ω, respectively. R_6, decade resistance variable from 1 to 10,000 Ω. C_1, C_2, C_3, condensers of 0.1, 0.05 and 0.02 μF., usable singly or in combination by means of switches K_1 to K_3. C_4, a bank of condensers, capacity variable from 0.01 to 6.0 μF. C_5, condenser of 0.1 μF. to limit polarization at electrodes. T_1, T_2, coreless induction coils, the first for stimulation, the second for controlling the grid in a signal circuit (not shown). T_3, T_3, a single transformer, operating from the 110 V. A. C. circuit and supplying 2.5 V. for heater filaments of tubes. K_5, switch for continuous stimulation; K_6, double throw switch for charging and discharging C_4.

Inset, upper left. Device for automatic control of stimulator. RT, tambour; MC, mercury cups. Explanation in text.

to about 1.5 sec. of uniform tetanization, by varying the capacity of the condenser C_4 and using, to charge and discharge it, the double throw switch K_6. During the discharge of C_4 the grid potential of A is raised above that of B for a period of time which varies directly with the capacity of C_4. On the graphic records shown the stimulation time is indicated in terms of capacity, 1 μF. corresponding to approximately 0.26 sec.

Automatic control of the circuit by the animal's respiratory movements is made possible by substituting, for the switch K_6, the device shown in the inset of Fig. 1. A tambour, connected to the intrapleural space, bears a long (30 cm.), light lever, pivoted near the middle. At each end of this lever a platinum wire projects toward a mercury cup below. The cups are adjusted at such levels that one contact is maintained throughout the expiratory pause, charging the condenser C_4. The change of intrapleural pressure during inspiration first breaks this contact and later closes the second, discharging the condenser. Vagal stimulation begins when the second contact is made. It may be terminated by a reflex expiration which breaks the contact, but in any event it cannot continue beyond the limit of time set by the capacity of C_4.

The "inspiratory" mercury cup is deep enough to avoid interference with the down-

ward movement of the lever. By raising or lowering this cup the discharging contact may be closed at an early or at a late stage of inspiration, as desired. The same device may be substituted for the single throw switch K_5, the "inspiratory" contact only being used. In that case stimulation, once started, continues until expiration breaks the contact. The coil T_2 (Fig. 1) is a duplicate of, and in parallel with, T_1. It has a resistance in series to limit diversion of current. It is used to control the grid of a third 885 tube, in an independent signal circuit. Some of our graphic records (Fig. 2, 3) were made before the automatic signal was added, and the periods of stimulation are not separately indicated.

Results

a. *The effect of varying the number of vagal volleys in a series.* Responses to vagal stimulation at a frequency of 80 per sec., recurring at the same stage in successive inspirations, are shown in Fig. 2. The duration of the stimulation period is increased progressively from 0.31 sec. (1.2 μF.) to 0.52 sec. (2.0 μF.). The latter duration means that some 40 volleys are delivered, a number sufficient in this instance to cut short all inspirations regularly.

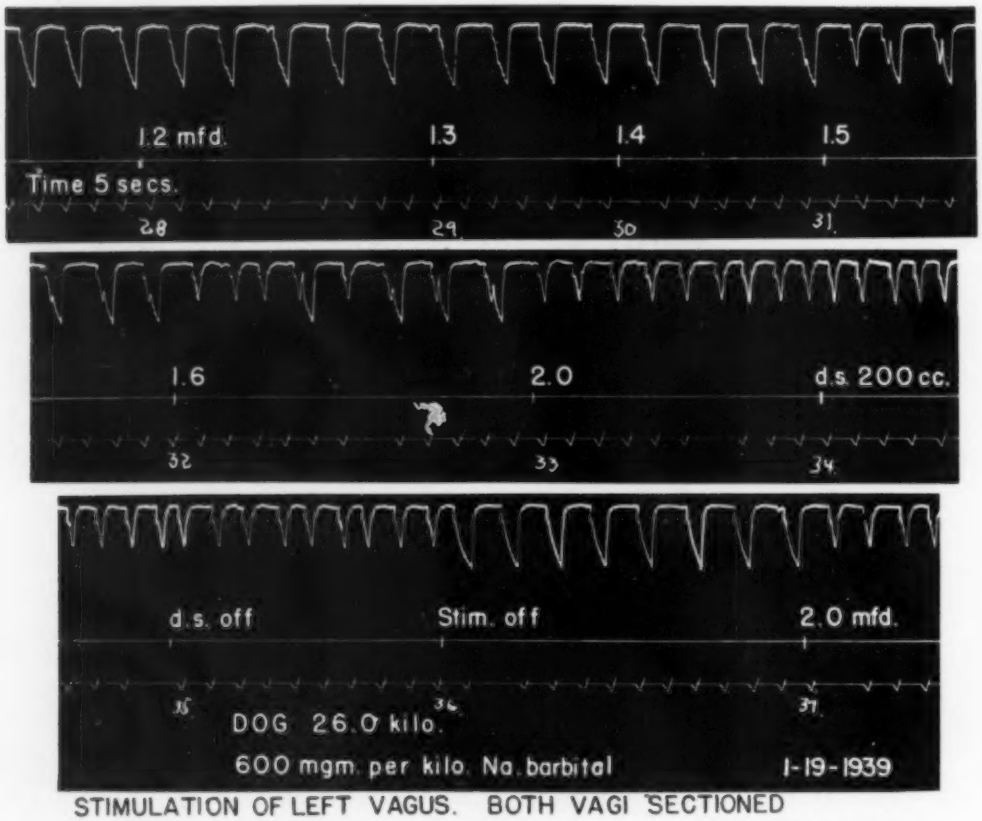


FIG. 2. Continuous record of respiration (intrapleural pressure, down stroke inspiration). Vagal stimulation, recurring at the same stage in successive inspirations, begins at first signal (top line) and continues to middle signal at bottom. Duration of stimulation periods progressively lengthened as indicated (1.0 μF. = 0.26 sec.). Showing (a) transient disturbance of inspiratory curve by a near-threshold series of volleys; (b) all or none effect on depth of inspiration; (c) inhibition still effective after dead space is increased.

Expiration goes to the normal level, and there is a definite, though abbreviated, expiratory pause. At 1.6 and 1.5 μF. there is a borderline effect. Some inspirations are cut short, others are merely interrupted by a rapid, incomplete expiratory movement after which the inspiration is resumed and goes on to full completion. The irregular responses in this near-threshold zone may perhaps depend upon accidental combinations of vagal impulses with other periodically recurring afferent influences, as from the carotid sinus.

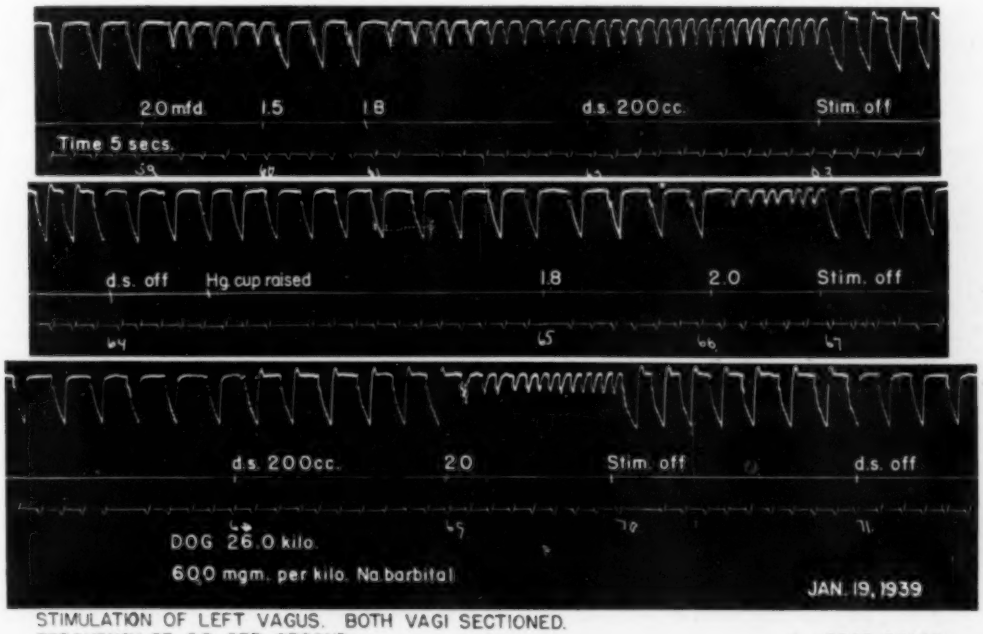


FIG. 3. Continuous record, same animal as in Fig. 2. First line, vagal stimulation for 0.39 sec. fails to cut short inspiration or to accelerate, but does both when the period is lengthened to 0.47 sec. The latter period of stimulation, however, is ineffective when applied earlier in inspiration (second line). Increase of dead space fails to raise the inhibitory threshold, although inspiration becomes slightly deeper (first and third lines). Note also that acceleration varies according to the stage at which inspiration is interrupted (compare first with second line).

With shorter periods of stimulation none of the inspirations are reduced in depth. The abortive expiratory movement becomes less marked, until, at 1.2 μF., it appears merely as a slight flattening of the inspiratory curve.

The abortive, partial expiration, caused by a near-threshold series of volleys, may mean either (i) brief reflex contraction of expiratory muscles, without an interruption of the inspiratory discharge, or (ii) momentary suspension of inspiratory activity followed by a "rebound" similar to that observed in spinal reflexes. By stimulating centrally the superior laryngeal nerve, it often is possible to keep an inspiration suspended at a mid-level for several secs. (Hillenbrand and Boyd, 1936). With the vagus, however, such an effect is in our experience always limited to a narrow range of

stimulation time,—usually somewhat narrower than in the example shown. It may be pointed out that the vagal "rebound" effect is obtained when the center is subjected to a near-threshold inhibition from which it is abruptly released, and presumably would not occur under natural conditions. The impulses from the stretch receptors do not cease sharply when inspiration stops, but die away gradually as the lungs are deflated (Adrian, 1933).

b. *The inhibitory threshold at different stages of inspiration.* The earlier vagal stimulation begins during inspiration, the longer is the stimulation period necessary to inhibit. Figure 3 shows the effect of raising the "inspiratory" contact. Before this change was made, stimulation for 0.47 sec. (1.8μF.) had been sufficient to cut short all inspirations regularly. Afterward

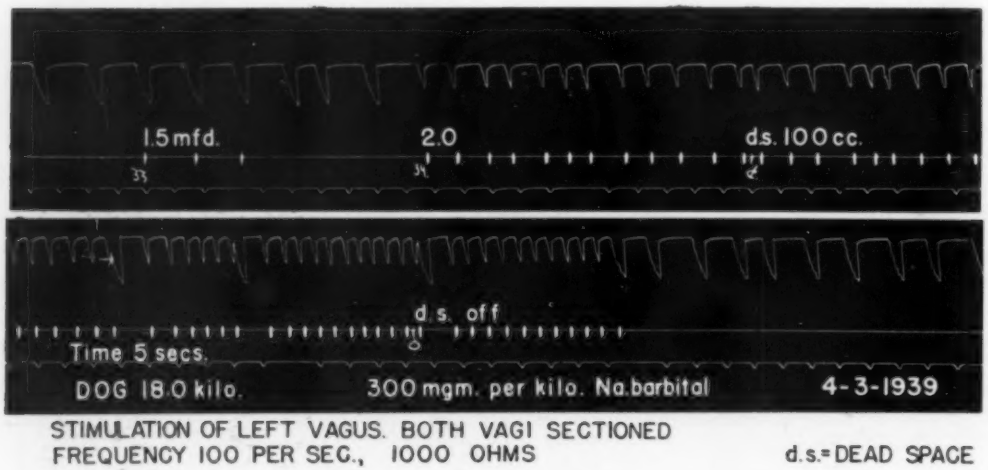


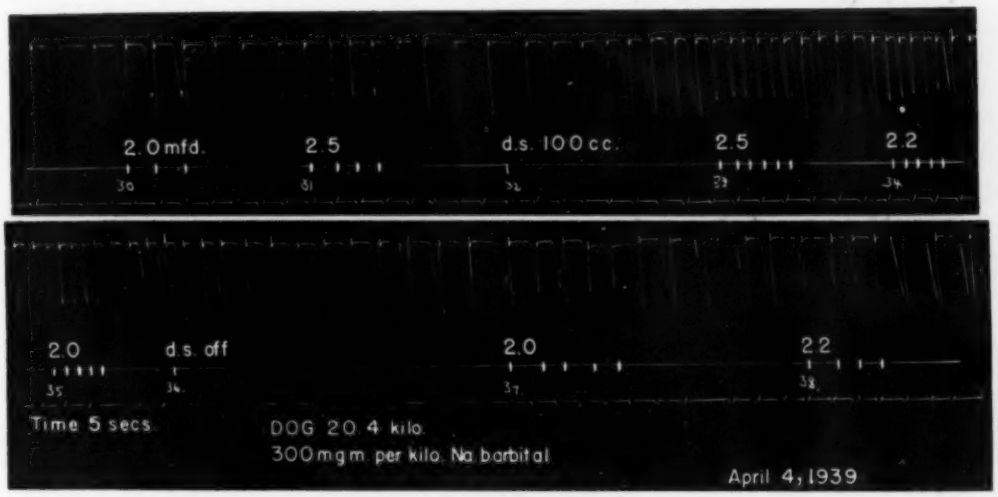
FIG. 4. Continuous record. With normal dead space vagal stimulation for 0.52 sec. cuts short all inspirations regularly. After increase of dead space an occasional inspiration breaks through (each stimulation period signalled).

the same stimulation period was found inadequate. Stimulation was then extended to 0.52 sec. (2.0 μF.) and inhibition again became effective. The lowering of threshold becomes quite marked during the final stages of inspiration. It is possible, of course, that the respiratory center is subjected during inspiration to inhibition from a higher center (Stella, 1938) or from receptors outside the lungs (Gesell, 1939); and that vagal stimulation becomes more effective, not because the center is inherently more susceptible, but because of the changing background of latent or subthreshold inhibition from these other sources. The behavior described, however, recalls the observation of Liddell and Sherrington (1925) that the crossed extensor reflex is more easily inhibtied in the latter part of its course than at the beginning.

c. *The effects of increased respiratory dead space.* With the tracheal cannula open directly to the outside air, we first determined the inhibitory threshold in the manner outlined in (a) above. A length of glass tubing (internal diameter 13 mm.), of 100 or 200 cc. capacity, was then attached

to the cannula. After a short time, respiratory stimulation was made evident by deeper and (usually) more rapid breathing. If the originally determined threshold stimulation was now applied to the vagus, recurring at the same stage of inspiration as before, the inspirations were still regularly cut short (Fig. 2, 3, 5). This procedure was followed with 11 dogs. Nine failed to show any elevation of the inhibitory threshold. With 2 animals (one instance is shown in Fig. 4) an occasional inspiration, after the dead space was increased, broke through the inhibition. On the other hand, the inhibitory threshold was sometimes actually lower after the dead space was increased (Fig. 5).

The failure of accumulating CO_2 to raise the inhibitory threshold was at first surprising, in view of the observations of Rice (1938). Rice applied



STIMULATION OF LEFT VAGUS BOTH VAGI SECTIONED.
100 per sec., 700 ohms. d.s. = DEAD SPACE

FIG. 5. Continuous record. Lowering of inhibitory threshold after dead space is inincreased. Vagal stimulation for 0.52 sec. cuts short all inspirations while the extra dead space is attached, fails to do so with normal dead space.

stimuli of uniform voltage and frequency to the central stump of one vagus (rabbit). The circuit was automatically closed at a fixed stage of inspiration and broken by expiration. With normal dead space, the inspirations were regularly inhibited at a relatively shallow level. With dead space increased the inspirations were still cut short, but not until they had been carried further toward completion.

Figure 3 of this paper shows a similar effect, though of small degree. After increase of the dead space the originally determined threshold stimulation of the vagus is still sufficient to inhibit, but the inspirations reach a slightly deeper level before they are cut short. The explanation apparently lies in the fact that under the influence of CO_2 the slope of the individual inspiration becomes steeper. This was noted by Rice, and is evident also on our records. When vagal stimulation begins at any given point on the

inspiratory curve, the final level reached will depend upon two factors. These are (i) the time required for temporal summation of the necessary number of inhibitory volleys, and (ii) the slope of the curve. When the latter becomes steeper there will be an increase of inspiratory depth even though the threshold of inhibition remains unchanged.

Rice's records (1938, p. 540) show an increase of depth exceeding any we have observed; but he used frequencies of stimulation somewhat lower than ours. The slower the frequency, the longer vagal stimulation must be continued in order to build up an effective inhibition; and the greater will be the difference in final level brought about by a given change in slope of the inspiratory curve.

d. *Concomitant changes in amplitude and rate.* Hillenbrand and Boyd (1936) noted that when an inspiration was inhibited by vagal stimulation, the ensuing expiratory pause was regularly shortened, provided care was used to avoid unnecessarily strong stimuli. This has been confirmed by Gesell, Steffensen and Brookhart (1937). The same acceleration of rhythm is manifest on the various graphs shown in this paper. It takes place too promptly to be attributed to changes in the chemical composition of the blood resulting from reduced ventilation. Moreover Adrian (1933) found a similar acceleration occurring when the inspiratory motor discharge was cut short by brief, properly timed inflation of the lungs (with vagi intact). Adrian suggested that the acceleration was an indirect consequence of inhibition, the center recovering more rapidly because it had not been allowed to discharge completely. The normal periodic activity of the stretch receptors, according to this view, has a dual effect; primarily it limits the amplitude of inspiration, but indirectly it keeps the rhythm accelerated.

Adrian's hypothesis seems to imply a reciprocal relation between the vagal effects upon amplitude and on rate. The earlier an inspiration is cut short the briefer should be the ensuing period of recovery. Hillenbrand and Boyd (1936) found that the acceleration does vary in degree according to the stage at which inspiration is interrupted. The effect upon rate becomes progressively less as the inspirations are allowed to go further toward completion. The same relationship is shown in Fig. 3 of this paper. The uppermost line shows a series of interrupted inspirations, with a marked acceleration of rhythm. The second line shows another series, cut short at an earlier stage and accompanied by a still greater acceleration.

Hammouda and Wilson (1935), however, hold that "augmentation of the rate of breathing cannot under any conditions be due to the stimulation of fibers carrying inhibitory impulses." They report that if the vagus be locally cooled to 8° C., the inhibitory fibers are selectively blocked. Continuous tetanization of the nerve then causes, not the usual reflex apnea, but an acceleration of breathing. Hammouda and Wilson believe that the afferent fibers responsible for this reversed effect are connected to pulmonary receptors; and that they are "augmentor" in function, tonically accelerating the respiratory rhythm.

The same authors, in a more recent paper, confirm the finding of Hillen-

brand and Boyd (1936) that breathing is accelerated by recurrent stimulation (limited to the inspiratory phase) of the uncooled vagus. This latter type of acceleration, they report, is lost when the vagus is cooled to 8°C. It therefore cannot be attributed to the "augmentor" fibers. They suggest (Hammouda and Wilson, 1939, pp. 522–523) that this particular type of acceleration is due to a third group of vagal afferent fibers, neither augmentor nor inhibitory, but having the same blocking temperature as the latter group.

We find, in agreement with Hammouda and Wilson, that cooling the vagus to about 8°C. abolishes simultaneously the inhibition of inspiration and the acceleration which accompanies it. But besides the identity of blocking temperature, the inhibitory and accelerator mechanisms have at least two other properties in common. First, the voltage threshold of the vagus is the same for both effects (stimulation being limited to inspiration). In preliminary tests at the beginning of each experiment we have used stimuli very weak at first but progressively strengthened. No acceleration ever has been observed from stimuli which were too weak to cut short inspiration. Acceleration regularly appears when the inspirations are inhibited, and not until then.

Second, if stimuli of adequate strength and frequency are applied, a a period of stimulation which is too short to interrupt inspiration also fails to accelerate. If the period of stimulation is lengthened, acceleration appears as soon as the inspirations begin to be cut short, not earlier. This is shown in Fig. 2, 3, 4, 5. It is of course conceivable that two distinct groups of afferent fibers might have the same blocking temperature, the same voltage threshold, and might furthermore require, to produce their independent reflex effects, summation of exactly the same number of volleys; but such a coincidence seems rather improbable.

We therefore conclude that the reciprocal changes of respiratory amplitude and rate, demonstrated by Adrian (1933), by Hillenbrand and Boyd (1936), and in this paper, are so intimately related that they must be due to a common mechanism. The change of rhythm is evidently dependent in some manner upon the reduced inspiratory discharge. The opposing arguments advanced by Hammouda and Wilson (1939) will be considered in detail in a later publication.

Summary

1. In dogs anesthetized with sodium barbital, and with both vagi sectioned, one vagus was stimulated centrally during inspiration. Adequate stimulation cuts short the current inspiration. This requires a certain threshold number of afferent volleys (voltage and frequency of stimuli being constant). The threshold number becomes less as inspiration advances.

2. Certain effects of a near-threshold series of volleys are described.

3. Graded reduction of inspiratory amplitude can be effected by varying the time at which the vagus is stimulated, but not by grading the number of inhibitory volleys. Vagal inhibition affects the respiratory center in an all or none manner.

4. Increase of the respiratory dead space does not raise the "inhibitory threshold" of the center. Inspiration becomes deeper, but this can be accaccounted for by a more rapid inspiratory movement, and by the time required for inhibition to be built up to an effective level.

5. The cutting short of inspiration, by the procedure described, is accompanied by an acceleration of rhythm. The amplitude and rate effects are related in the following ways:

(a) The voltage threshold of the vagus is the same for both.

(b) Both effects require temporal summation of a series of vagal volleys. The minimum number of volleys required to accelerate is also the threshold number for inhibition of inspiration.

(c) Both effects are simultaneously lost when a cold block of the vagus is gradually induced.

(d) The acceleration varies in degree according to the stage at which the inspirations are cut short.

6. It is concluded that the effects upon rate and amplitude are probably due to a common afferent mechanism, the acceleration being dependent upon the reduced motor discharge.

7. A stimulating circuit is described. It permits uniform tetanization for controllable short periods (0.01 to 1.5 sec.), frequency and stimulus strength being independently variable. The circuit can be automatically governed by respiratory changes of intrapleural pressure.

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CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF VARIOUS AFFERENT CONNECTIONS

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Laboratories of The Rockefeller Institute for Medical Research, New York

(Received for publication May 26, 1939)

INTRODUCTION

THE IMPORTANCE of the afferent fiber connections as a basis for functional localization in the cerebellum has been recognized since the work of Ingvar in 1918. From that time on extensive comparative anatomical investigations (Larsell, 1937) and ablation experiments (see Fulton and Dow, 1937, for recent summary) have supported a cerebellar division based on afferent connections. The importance of obtaining by still another method more specific information with respect to the afferent fibers is obvious. The present work is an attempt to confirm by an oscillographic method previously known afferent connections and to obtain additional information on the pontocerebellar and olivocerebellar connections. This method is advantageous for several reasons: (i) It gives direct evidence of the functional importance of the previously known afferent connections; (ii) it is not limited to myelinated fibers, as is the classical method of Marchi; and (iii) it is capable of easily establishing the presence of connections through one or more synapses.

HISTORICAL REVIEW

In 1912, Beck and Bikeles with the use of a sensitive galvanometer studied the effects on the cerebellum of single induction shocks applied to the sciatic nerve or to the nerves of the brachial plexus, and also the effects of thermal stimulation of the cerebral cortex. No pictures of the responses were given and only a list of the galvanometric deflections was presented in tabular form. When the peripheral nerves were stimulated, responses were found predominantly on the vermis of the cerebellum and not usually on the hemispheres. The nerves of the upper and the lower extremities were apparently equally capable of producing the effect, and as far as the responses were analyzed, the extent of the responsive area was identical following stimulation of the nerves of either the upper or the lower extremities. Thermal stimulation of the sensory motor cortex of the cerebral hemispheres gave a galvanometric deflection on both hemispheres, predominantly on the crossed one and occasionally on the vermis as well. There was no significant difference in the cerebellar response, whether the arm or the leg area was stimulated.

Camis in 1919 observed from the recordings of a string galvanometer that there was a change in the electrical activity of the cerebellum following mechanical or rotational stimulation of the vestibular apparatus. The change was not seen when the leads were placed on any part of the exposable cortex of the cat, but it could be observed when they were placed one in the fastigial nucleus and the other in the dentate nucleus, after removal of the overlying cortex. Price and Spiegel (1937) during rotation obtained an increase in the amplitude and frequency of the characteristic cerebellar activity in the most caudal part of the vermis (uvula). No previous studies of cerebellar action potentials following electrical stimulation of the eighth nerve or stimulation of the pons or inferior olivary nuclei have been reported.

Attempts (Dow, 1938) to modify the "spontaneous" activity of the cerebellum by tetanic stimulation of peripheral nerves failed to produce any effects that could not be explained as secondary to a rise in blood pressure.

4. Increase of the respiratory dead space does not raise the "inhibitory threshold" of the center. Inspiration becomes deeper, but this can be accaccounted for by a more rapid inspiratory movement, and by the time required for inhibition to be built up to an effective level.

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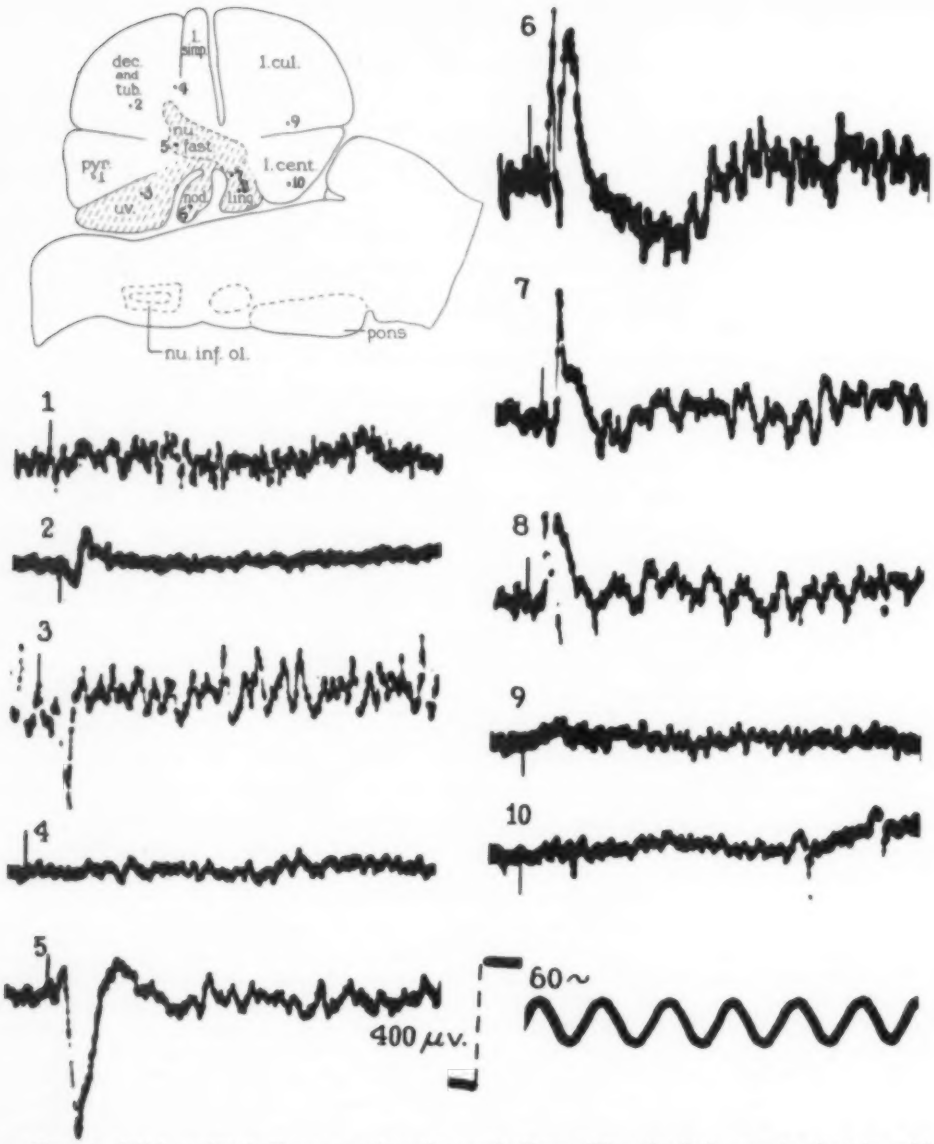

FIG. 1. Mid-sagittal diagram of cat's cerebellum. The shaded area is that in which responses to eighth nerve stimulation were found. The numbered points give the positions from which the oscillographic records were obtained in the experiment illustrated. The inactive points, 9 and 10, were active when the sciatic nerve was stimulated. Records taken with bipolar needle electrodes with bared tips, 1 mm. apart.

dec. and tub.,	declive and tuber vermis
l. cent.,	lobulus centralis
l. cul.,	culmen
l. simp.,	lobulus simplex
ling.,	lingula
nod.,	nodulus
nu. fast.,	fastigial nucleus
nu. inf. ol.,	inferior olivary nuclei
pons,	pons
pyr.,	pyramis
uv.,	uvula

MATERIAL AND METHODS

Forty decerebrated cats were used in the present investigation. Stimulation of the nerves was effected by means of condenser discharges of short duration. The stimulus was delivered through an audio-transformer to reduce the shock artifact. After transection, the nerves were placed in a glass stimulating electrode which was buried in the limb muscles. The cord was stimulated directly by applying a small Lucite crescent-shaped plate, in which two wires had been embedded, directly to the lateral surface of the cord. In order to eliminate as much as possible the effects of this stimulation on parts of the cord other than the spino-cerebellar tracts, the cord was frequently sectioned transversely below the site of stimulation and longitudinally for 1.5 to 2 cm. at the level of the site of stimulation. The eighth nerve was stimulated by exposing it in the internal auditory meatus and applying thin silver wire electrodes directly to the nerve trunk. The pons and the inferior olivary nuclei were stimulated through two steel needles 1 mm. apart and insulated except at their tips.

A differential amplifier (Toennies, 1938) and a cathode ray oscillograph were employed. For surface leading the ground was placed on the animal head holder, the active grid on the cerebellum, and the inactive grid on the skull. This "unipolar" leading gave good localization on the surface. When a needle electrode was used to lead from points within the cerebellum, potentials of small amplitude were occasionally led from lobes that gave no surface response. Movements of the needle electrode as great as several millimeters did not change the amplitude, sign or shape of these responses. Bipolar leads in the same positions revealed no appreciable potential gradients. Consequently the potentials recorded were believed to arise at relatively remote loci. In order to differentiate locally produced potentials from those arising at a distance, all regions of the cerebellum were explored with bipolar leads consisting of two needles insulated except at their tips and separated by one mm.

The deep parts of the cerebellum were explored by systematically moving the bipolar electrodes to different points in a particular vertical plane by means of a mechanical manipulator, the movements of which could be measured accurately. The positions of the electrodes were identified by the distance from an electrolytic lesion made at some point in the path of the needles and located at the end of the experiment in thin sections cut from the brain after it had been fixed in formalin. After some experience it was found possible to use physiological landmarks as aids in locating the electrodes in the course of an experiment.

Experimental Results

Stimulation of the eighth nerve. Responses to stimulation of the eighth nerve were found in the flocculonodular lobe, the uvula, lingula, and fastigial nucleus (Fig. 1). The potentials were obtained by using bipolar needle electrodes and systematically exploring all the lobes. The latency—the interval between the shock artifact and the beginning of the first potential clearly separate from any "spontaneous" activity—in the midline cerebellar structure was 4.5. msec. In one experiment with the lead electrodes at the base of the nodulus, in addition to the response beginning at 4.5 msec. there was a spike-like potential having a total duration of only 1.8 msec. This potential began 0.6 msec. after the shock artifact and probably was produced by the vestibulocerebellar nerve fibers. It is known from degeneration experiments that in the cat primary myelinated vestibular fibers enter the cerebellar cortex at this point (Ingvar, 1918; Dow, 1936). There were true cerebellar action potentials in the homolateral flocculus after a latency of 3 msec. It is impossible to know whether or not earlier potentials, such as the spike recorded at the base of the nodulus, were present, because in some experiments (Fig. 2) the shock artifact when leading from the homolateral flocculus interfered with the record for as long as 2.5 msec. A response

was seen in the contralateral flocculus in the one experiment in which the contralateral lobes were explored.

These lobes and nuclei were the only ones from which potentials could be obtained when bipolar needles were used. Occasionally potentials of low amplitude resulted from other lobes with unipolar leads; but their size and

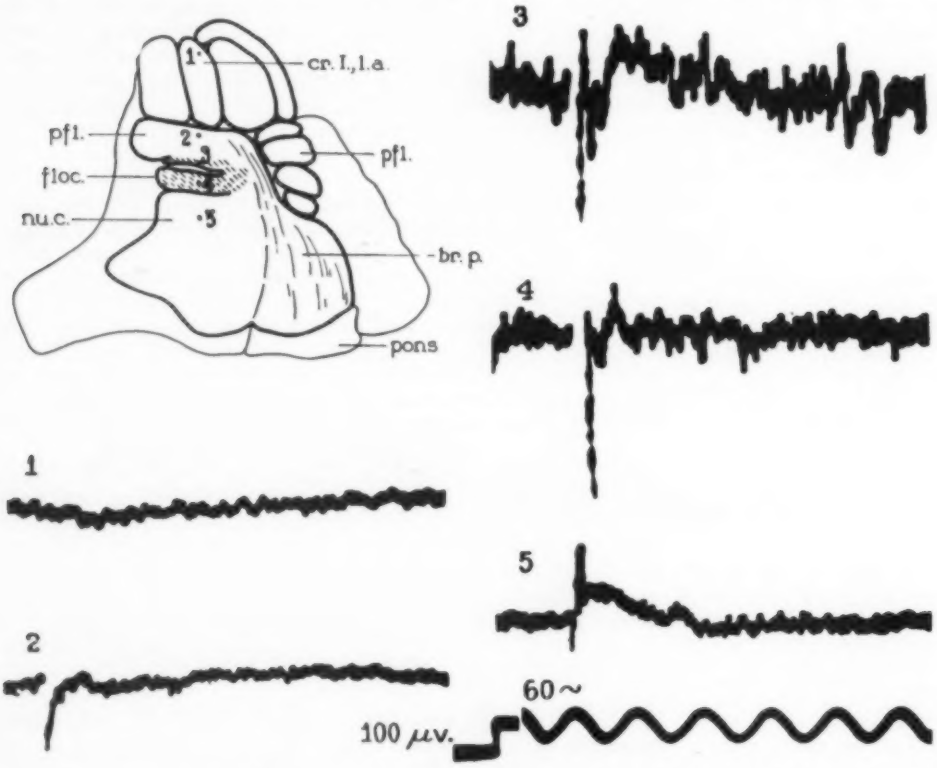

FIG. 2. Para-sagittal diagram of the cat's cerebellum with points indicating the position of the leads in an experiment in which the eighth nerve was stimulated. Responses were obtained in the flocculus, but not in the lobulus ansiformis and paraflocculus. Point 5, in dorsal cochlear nucleus. Records taken with bipolar needle electrodes with bared tips, 1 mm. apart.

br. p.,	brachium pontis
cr. I., l. a.,	crus I, lobulus ansiformis
floc.,	flocculus
nu. c.,	dorsal cochlear nucleus
pfl.,	paraflocculus

the failure to change in form with movement of the needle indicated that they were not produced in the region of the leads.

The facts reported above would not in themselves differentiate between responses to stimulation of the vestibular division of the eighth nerve and of the auditory division. That the responses are caused by stimulation of the auditory division is unlikely on anatomical grounds, and from the work of Camis (1919) and of Price and Spiegel (1937) we have evidence that physio-

logical stimulation of the vestibular apparatus can result in an alteration of the electrocerebellogram. In the course of the present work there was no effect from sound stimulation in any part of the cerebellum, including the lobes giving the most marked responses to vestibular impulses, although

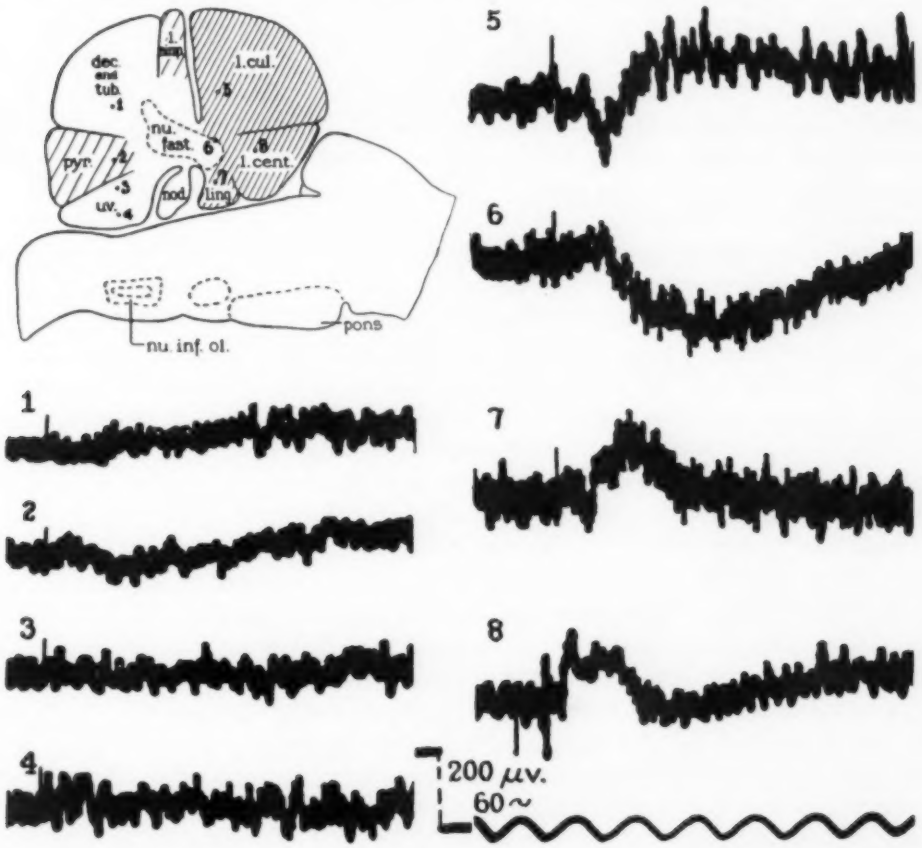

FIG. 3. Mid-sagittal diagram of cat's cerebellum. The shaded area shows lobes responding to stimulation of spinal nerves. Numbers of points and records correspond; taken from a single illustrative experiment. Note that the principal responses occur throughout the anterior lobe. Record 2 from pyramis shows a slight effect, considerably less than usual. Records taken with bipolar needle electrodes with bared tips, 1 mm. apart. Normal activity of some of the silent areas was controlled by stimulation of the pons in this experiment. Vestibular stimulation used as control of activity of the uvula and nodulus in other experiments.

responses in the acoustic tracts and nuclei to both sound and electrical stimuli were regularly obtained.

The presence of potentials arising locally in the flocculonodular lobe, the lingula, uvula, and the fastigial nucleus, and their absence in other lobes and nuclei, are in accord with the anatomical findings of Ingvar (1918) and of Dow (1936) on the distribution of primary vestibular cerebellar fibers in the cat. If the distribution is the same in the cat as in the rat, it is also in

accord with the location of the terminations of the secondary vestibular fibers (Dow, 1936).

Stimulation of the spinal nerves or spinal cord. The sciatic nerve was chosen for the majority of the 16 experiments of this group. When the nerves of the upper extremity were stimulated, the median and ulnar nerves were used. The cord was stimulated directly in the mid-dorsal region. The entire anterior lobe, the lobulus simplex, pyramis, and occasionally the lobulus paramedianus responded to stimulation of the spinal cord or its nerves. The declive and tuber vermis, the lobulus ansiformis, paraflocculus, uvula, and flocculonodular lobe gave no potentials in response to spinal stimulation (Fig. 3). The effect was bilateral, but more marked on the homolateral side. When recorded from surface electrodes, the potential was frequently complex, but the predominant potential deflection was surface positive. The beginning of the potential resulting from stimulation of the sciatic nerve was usually seen after a latency of from 8 to 13 msec., depending upon the experiment and the part of the cerebellum from which the potentials were led. In a few experiments high amplification revealed an earlier surface positive wave, in one instance after only 4.5 msec. Because this early wave was most frequently seen when the lead electrode was near the peduncles and because of its short latency, it may be a record of the potentials of the incoming spinocerebellar fibers. The latency following stimulation of the nerves of the brachial plexus varied from 3.5 to 5 msec., depending upon the experiment and the part of the cerebellum from which potentials were led. When the cord was prepared so that the spinocerebellar fibers were stimulated directly in the mid-thoracic region, the first potential, always surface positive, was seen after a latency of only 1 msec.

The lobes responding were the same, regardless of the site of stimulation. Indeed, in the experiment shown in Fig. 4 one could compare point for point throughout the entire exposable cortex the effect of stimulation of the nerves of the upper extremity with that of the lower. Not only were there potentials in the same lobes, but frequently even the form of the potential was remarkably similar. This was true even when the animal was under ether anesthesia deep enough to eliminate any possible reflex effects from one extremity to the other. The finding suggests that the same elements may be activated by stimuli arising in either the upper or the lower extremity. The distribution and complexity of the response at a given point were unchanged when the reflexes were abolished by curare. If the animal was anesthetized with dial and not decerebrated, the distribution of the spinal responses was the same, in so far as the exposure allowed an exploration of the surface of the cerebellum. No physiological stimulation has been attempted in the present experiments. Stimulation of the saphenous nerve, which is cutaneous in distribution, resulted in a cerebellar potential, which was still present after all reflexes had been abolished by a large dose of curare.

Responses in the "spinal parts" of the cerebellum may be obtained from stimulation in the dorsal reticular formation (Fig. 7). Whether they are

the result of the stimulation of nuclei situated there, or of fibers passing through the brain stem on their way to the cerebellum, cannot be determined from the data at hand.

Stimulation of the pons. Responses from the pons were obtained in the

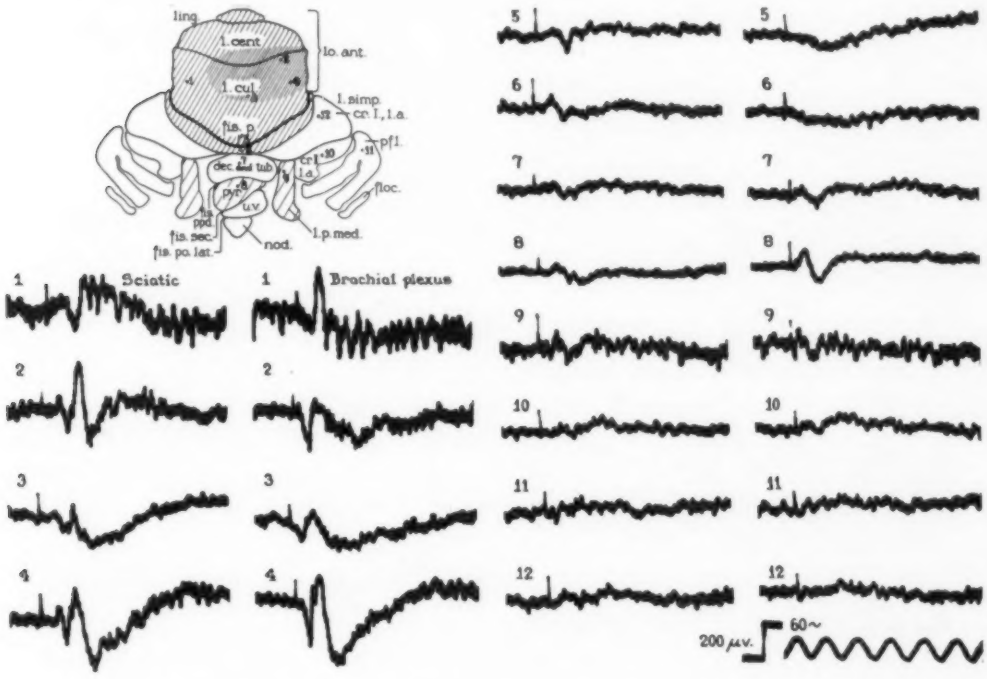

FIG. 4. Schematic diagram of folial pattern of cat's cerebellum. The shaded area shows the lobes responding to stimulation of nerves of right upper and lower extremities. The degree of shading is a rough index of the amplitude of the responses and shows diagrammatically the tendency for the greatest response on homolateral side. Records from a single experiment allow a comparison, point for point, of the effect of stimulation of nerves of upper and lower extremity. Note the correspondence in area responding and striking similarity of potential (records 3 and 4). Note apparent increase in "spontaneous" activity on contralateral side after nerve stimulation, record 1. "Unipolar" cotton wick electrode used.

cr. II, l. a.,	crus II, lobulus ansiformis
fis. p.,	fissura prima
fis. po. lat.,	fissura postero-lateralis (Larsell)
fis. sec.,	fissura secunda
fis. ppd.,	fissura prepyramidalis
lo. ant.,	anterior lobe
l. p. med.,	lobulus paramedianus

lobulus ansiformis, paraflocculus, lobulus paramedianus, and the declive and tuber vermis (Fig. 5). At times the culmen lobulus simplex and pyramis also gave responses of a somewhat lower amplitude. The response usually consisted of a surface positive potential of spike-like proportions which appeared out of the shock artifact and was followed by a much longer lasting

potential, usually surface negative. The slower potential was occasionally surface positive, especially when a record was made from lobes caudal to the fissura prima. When no potentials were obtained from the surface, bipolar needles thrust below the surface likewise revealed no potentials

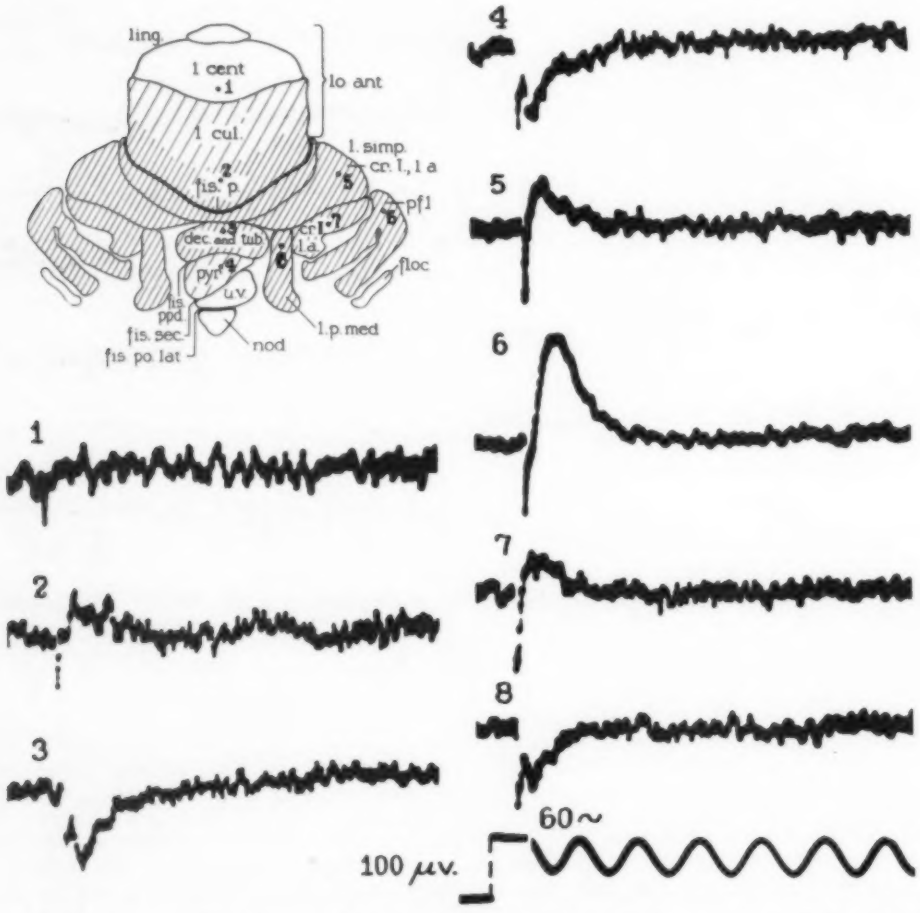

FIG. 5. Schematic diagram of folial pattern of cat's cerebellum. The shaded area gives the lobes responding to pontine stimulation, and the intensity of the shading furnishes a rough index of the relative amplitudes of the responses ordinarily encountered. Numbers of points and records correspond; taken from single illustrative experiment. "Unipolar" cotton wick electrode used. Note that almost the whole of the exposable cortex in the cat shows a response to stimulation of the pons.

(Fig. 6); but unipolar needles revealed low potentials that, as in the experiments in which the vestibular nerve was stimulated, probably represented action at a distance.

As yet no conclusions have been reached regarding the differential distribution of fibers from various parts of the pons, for example, the pre- and post-trigeminal parts of the pons which, as indicated by comparative

evidence (Abbie, 1934), may have a different distribution. The experiment shown in Fig. 6 suggests that the pontine projection to the anterior lobe arises only in the most posterior parts of the pons. A stimulus more localized than has been desirable in these experiments may bring some evidence to bear on this point. Activation of the pontocerebellar projection synaptically, by cerebral cortical stimulation, has not been attempted; in view of the

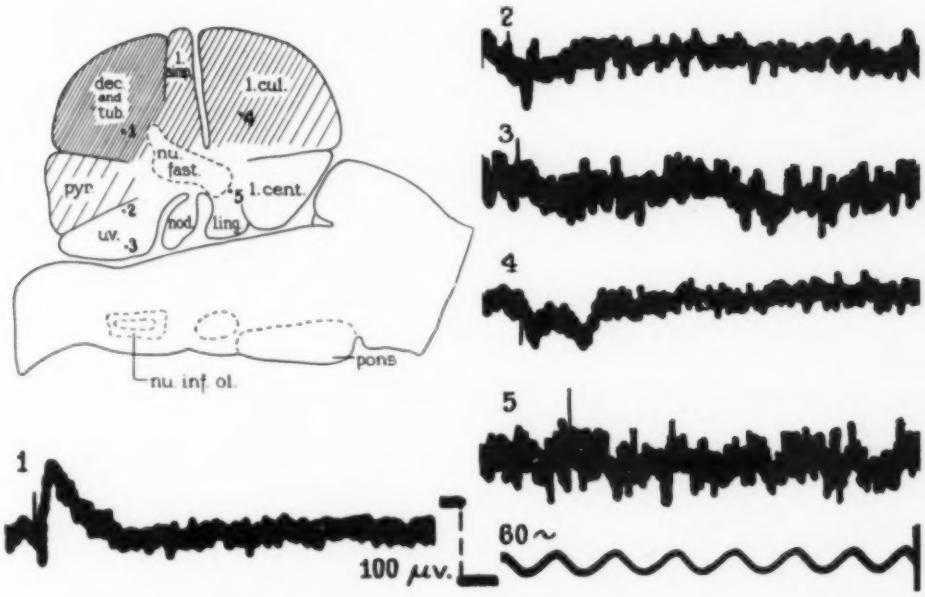

FIG. 6. Mid-sagittal diagram of cat's cerebellum. The shaded area shows the lobes responding to stimulation of the pons. The degree of shading gives a rough index of the relative amplitudes of the usual responses. Numbers of points and records correspond; taken from single illustrative experiment. Point 4 gave no response when the stimulating electrode was in the rostral part of the pons, but gave the response seen in record 4 when the caudal part of pons was stimulated.

effects of thermal stimulation reported by Beck and Bikeles (1912) it should yield interesting results.

Stimulation of the inferior olivary nuclei. Stimulation of this region gives responses in all parts of the cerebellum including the flocculus. The responses of greatest amplitude were found in the contralateral hemisphere, but homolateral responses were also seen (Fig. 7).

The responses, usually surface negative, were often preceded by a surface positive potential and were seen after a latency of 5 to 5.5 msec., depending upon the experiment. Occasionally the relation of the surface lead electrode to a fissure had great influence upon the sign of the potential. With identical lead positions there occurred a characteristic difference in the sign of the responses, depending upon whether the dorsal reticular formation, or the inferior olive, was stimulated. Movement of the stimulat-

ing electrodes by only 3 mm. was sufficient to change not alone the sign of the response, but also the extent of the cortex responding and the recovery characteristics.

The olive response was exceedingly sensitive to conditioning by previous activity. The response to the second of two maximal shocks was often com-

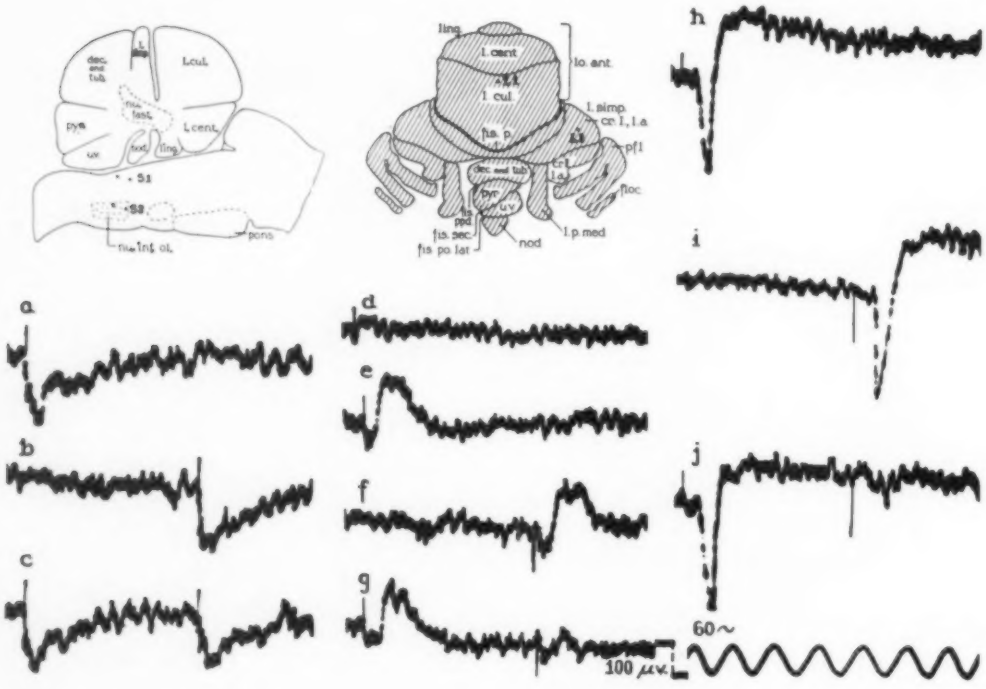

FIG. 7. Mid-sagittal section of cat's cerebellum showing position of stimuli, S_1 and S_2, and a folial pattern showing the position of the leads L_1 and L_2. *a*, *b*, *c:* Stimulus S_1 in dorsal reticular formation and lead L_1 on ventral part of culmen. Responses surface positive. *a*, Conditioning alone; *b*, testing alone; *c*, both. Note that the inhibition of the second response is very slight. *d*, Stimulus S_1 and lead L_2 on the lobulus ansiformis. Note absence of response. *e*, *f*, *g:* Stimulus S_2 in inferior olivary nuclei, lead L_1. Responses now surface negative. *e*, Conditioning alone; *f*, testing alone; *g*, both. Note marked conditioning. *h*, *i*, *j:* Stimulus S_2, lead L_2. Almost complete inhibition by a conditioning action 65 msec. before the test. "Unipolar" cotton wick electrode used for the leads.

pletely suppressed when the second shock came 70 msec. after the first; and the periods of subnormality lasted as long as 5 sec. Leads from the olive showed that the conditioning was in the olive itself and not in the cerebellum. It would appear that a stimulus in the region of the olive or just rostral to it excites synaptically the whole of the homolateral nucleus. The interconnections within the olive are not well-known. Occasionally there was a response in the cerebellar cortex that was not completely inhibited by a previous shock, which suggested that the olivocerebellar fibers, or their cells of origin, were stimulated directly. These less readily inhibited

responses were not widely distributed over the cerebellum, as were the more readily inhibited ones. It is possible that by taking advantage of this method of separation of direct and indirect excitation of the olivocerebellar connections, and by using more localized stimulation, details concerning the point to point relationship between parts of the olivary nuclei and specific lobes of the cerebellum may be worked out oscillographically.

The difference in the responses led from the same point when two totally different afferent systems are stimulated, as for instance the inferior olivary nucleus and the spinocerebellar tract (Fig. 7), suggest that different elements of the cerebellar cortex, or different pathways in the cortex, are involved. A possible anatomical basis for the difference lies in the presence of two systems of afferent fiber connections to the cerebellar cortex, namely the mossy fibers with their connection to the granular cells and thence to the Purkinje cells, and the equally important climbing fibers that have direct synaptic connections with the Purkinje cells.

Although the present data do not identify the elements within the cerebellar cortex responsible for the potentials, evidence does seem to point to the cerebellar neurons as the chief source of the potentials, rather than to the afferent axons and their endings. The evidence rests upon the fact that the synaptic region of neurons is much more dependent upon readily available oxygen than are nerve fibers. The cerebellum was rendered ischemic by clamping the carotid arteries in a preparation in which the vertebral arteries had previously been ligated. During the clamping, artificial respiration was kept up in order to prevent a falling off of the oxygen supply of the spinal cord because of depression of the respiratory center. Twenty seconds after the clamps were applied, the "spontaneous" activity began to be affected and at the same time there was a decrease in the response to sciatic stimulation. At the end of 30 sec. when the spontaneous activity had disappeared, the response was further reduced, but it did not disappear altogether until 45 min. had elapsed.

The response in the cerebellum to stimulation of the cerebellar tracts in the spinal cord was depressed in a similar manner by asphyxia or ischemia. In this instance, however, a potential small in comparison with the original one could still be lead from the anterior lobe after 15 min. The potential undoubtedly originated in tract fibers, and it served as a contrast to the neuron potentials which were depressed in a period measured in seconds.

The arrival of a single afferent volley and the response of the cerebellar cortical neurons have remarkably little effect upon the background or the so-called "spontaneous" activity which is characteristic of the cerebellum and which is known to vary in amplitude and frequency as the effect of the cerebellum upon muscular tonus varies (Dow, 1938). However, in a few experiments in which for some reason the spontaneous activity was decreased, the arrival of an afferent volley set off a short chain of rhythmic waves at the usual frequency of 150 to 250 per sec. If was a regular observation following stimulation of a spinal nerve that the response on the contra-

lateral side of the anterior lobe, although not as large as that on the homolateral side, was associated with a definite increase in the amplitude and frequency of the background activity (Fig. 4 1). This effect might be caused by fractionation of the response on the edge of the responding field, or by the activation of laterally conducting elements within the cerebellar cortex. In no instance was there any suppression of the activity following a single afferent stimulation. These observations, as far as they go, are in agreement with those of Price and Spiegel (1937) who found an increase in the amplitude and frequency of the cerebellar potentials as recorded from the uvula during rotation of the animal.

Conclusions

1. Single shock electrical stimulation of afferent connections to the cerebellum results in a response of the neurons of the cortex in the lobes with which the respective afferent system has connections.

2. Stimulation of the eighth nerve in the unanesthetized decerebrated cat results in an electrical response in the cerebellum, limited to the flocculonodular lobe, the lingula, uvula, and the fastigial nucleus.

3. Stimulation of the spinal nerves, or of the spinocerebellar tract in the mid-thoracic cord, produces cerebellar action potentials limited to the anterior lobe, the lobulus simplex, pyramis, and occasionally the lobulus paramedianus. No difference in location of the response depending upon the site of stimulation was seen, except for a response of greater amplitude on the homolateral side.

4. Stimulation of the pons sets up cerebellar action potentials limited to the middle lobe of the vermis (Ingvar), the lobulus ansiformis, lobulus paramedianus, paraflocculus, and pyramis. Occasionally potentials are found also in the dorsal part of the culmen.

5. A stimulus in the region of the inferior olivary nuclei excites synaptically the olivocerebellar connections and produces cerebellar action potentials in all the lobes of the cerebellum. The responses of greatest amplitude are found when leading from the contralateral lobulus ansiformis.

6. In similar afferent systems, such as the nerves of the upper and the lower extremities, the responses led from an identical point may be strikingly similar. With dissimilar afferent systems, such as the inferior olive and the spinal system, potentials of different sign and shape may be led from the same point. The question is raised whether this dissimilarity of potential depending upon the source of the afferent stimulation may not be attributable to a difference in the anatomical connections within the cerebellar cortex.

7. It is thought that these action potentials are due to activity of the neurons of the cerebellar cortex and that they are not potentials of the axons or endings of the afferent fibers. In most instances the activity resulting from single shocks changes to a remarkably small degree the background activity which is characteristic of the cerebellar cortex. Under certain con-

ditions, however, this activity may be initiated or increased by a response to an afferent stimulation. In no instance has a decrease been observed.

It is a pleasure to express my deep gratitude to Dr. Herbert S. Gasser and to the members of his Laboratory for advice and helpful criticism during the course of this work.

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CHANGES IN RETINAL EXCITABILITY DUE TO POLARIZATION AND SOME OBSERVATIONS ON THE RELATION BETWEEN THE PROCESSES IN RETINA AND NERVE*

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INTRODUCTION

THE RETINA would seem to be a useful preparation for studying the effect of a polarizing current on the excitability of sensory neurones relaying impulses to other neurones. We have undertaken such experiments and, although their interpretation is difficult, the results are clear cut and at least give some information about the relation between the processes in the retina and the optic nerve. Because of their significance for the problem under discussion some observations on the effect of antidromic impulses into the

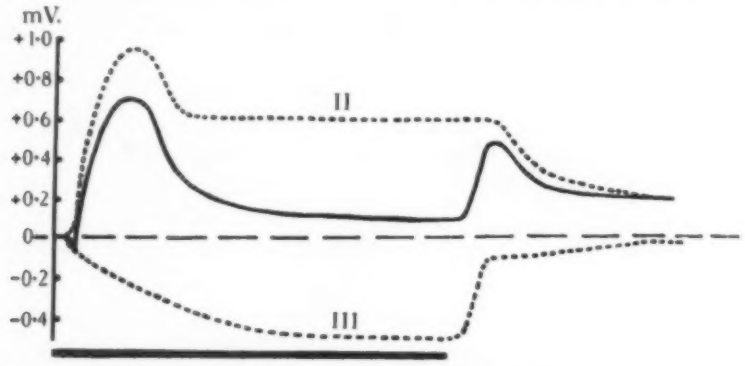

FIG. 1. Analysis of light-adapted frog's electroretinogram according to Granit and Riddell, 1934. See text.

optic nerve and on the latent period of the retinal and nerve processes have been added.

For the purpose of this paper we can neglect the slow secondary rise of the retinal electrical response, known as P I. We are here chiefly interested in the two remaining potentials of opposite sign, both of which are directly connected with the states of excitation and inhibition set up by light. Actually this restriction only means that we are dealing with the light-adapted frog's eye since the slow component P I requires dark adaptation.

The electroretinogram of the light-adapted frog's eye is shown in the diagram of Fig. 1, which also illustrates its analysis into components. The off-effect is solved as an interference phenomenon due to the negative P III returning to the base line faster than the positive P II (Granit and Riddell,

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1934). Later work by Granit and Therman (1937) has suggested the necessity of introducing a renewed rise of P II at cessation of illumination. For the cat's eye, where the off-effect is merely a retardation of the fall towards the base line at "off," the interference-theory still would seem to be valid (Granit, 1933). Further details may be found in recent publications by Hartline (1937, 1938), Granit (1938), and Granit and Therman (1937). It may further be added that the diagram of Fig. 1 refers to the standard leads: cornea, lens, or vitreous body←→back of the bulb. Neither P II nor P III can be explained as the sum total of rapid spikes of impulses (see, *e.g.* Granit, 1938, Therman, 1938).

METHODS

The amplifying and recording technique used in several earlier contributions to similar problems has been employed. A double cathode ray, one directly coupled push-pull ampli-

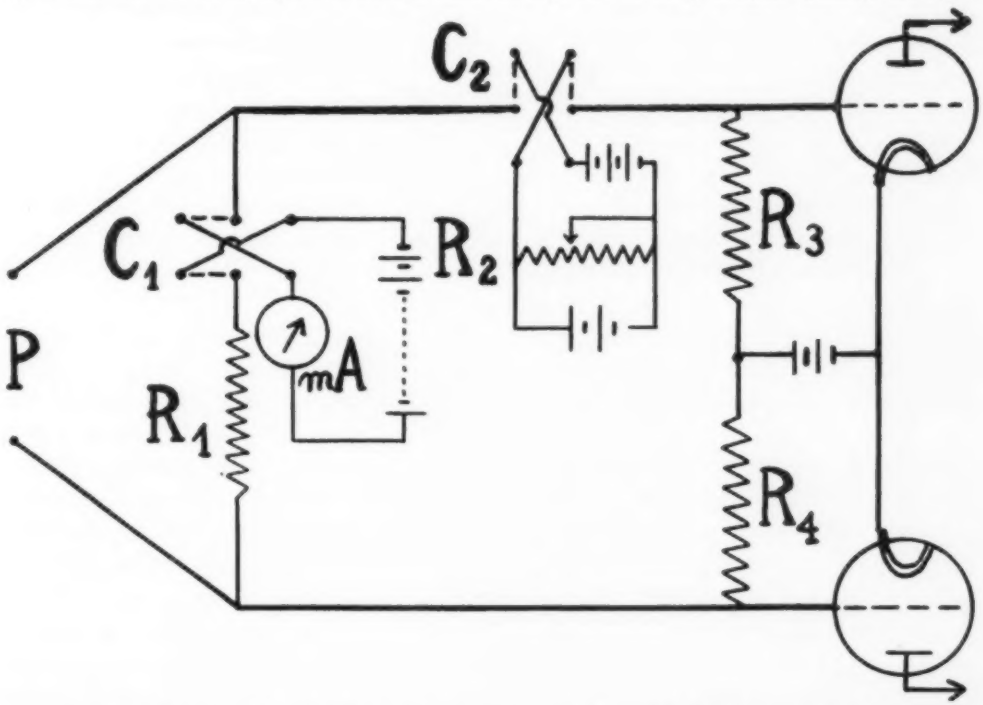

FIG. 2. Diagram of polarizing and compensating circuit in the input of the amplifier. P preparation (frog's retina) between electrodes used for leading off and for polarizing. R_1 0.5 MΩ; R_2 1000 Ω potentiometer; $R_3 = R_4$ 0.5 MΩ; mA milliammeter; C_1 and C_2 commutators.

fier, and two balanced condenser coupled amplifiers have been available for leading off from silver-silver-chloride electrodes on the excised opened frog's eye (standard lead) and its optic nerve. The pair of balanced amplifiers was used for simultaneous records from retina and nerve, the directly coupled instrument when it was necessary to obtain an undistorted picture of the electroretinogram.

Polarization across the standard retinal leads offered no problem when records were taken from the optic nerve. But with the eye itself between the leading-off electrodes steps had to be taken to avoid blocking of the amplifier by the polarizing current. In some ex-

periments this was done by putting a pair of 4μF condensers in series with the input, but a better method enabling use of the properties of the directly coupled amplifier was found to be the one illustrated in Fig. 2. Also in this case silver-silver-chloride electrodes were used though some experiments also were carried out with calomel half-cell electrodes.

In Fig. 2, illustrating the input of the push-pull amplifier, the polarizing current containing the commutator C_1 traverses the retina over the large resistance R_1 smoothing out changes in the resistance of the preparation (about 10,000 Ω). The circuit compensating for the drop of potential across P contains batteries, the commutator C_2 and potentiometer R_2 for balancing out the amplifier. The two commutators C_1 and C_2 are switched over simultaneously. As the effect of polarization on the retinal response is reasonably constant for several minutes, there is ample time for adjusting the sensitivity of the amplifier and waiting for the initial drift caused by the polarizing current to cease before the eye is illuminated.

Results

1. *Leading off from the nerve.* The input of the amplifier was connected to electrodes on the whole nerve and the excised opened eye stimulated by light during the passage of a galvanic current across the standard leads. For reasons to be explained below it is important to note that the opened bulb should not be allowed to become dry. "Inside cathode," "inside anode" means that the cotton wick touching the fluid inside the bulb is cathode and anode respectively.

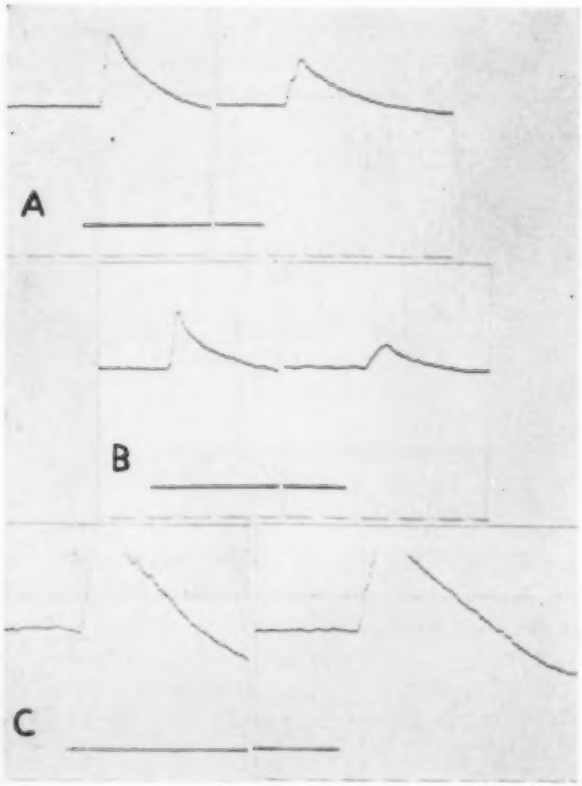

FIG. 3. Effect of polarization on the discharge through the optic nerve. Illumination for 10 sec. marked on film above time in 1/5 sec. Relatively low sensitivity in order to obtain the main deflexions at onset and cessation of illumination. A, normal on- and off-effects; B, "inside anode," polarizing current 0.4 mA; C, "inside cathode," polarizing current 0.4 mA. Amplifier in this case thrown out of its working range by the large deflexions at "on" and "off."

With this arrangement easily repeatable results were possible. For galvanic currents between 0.3–1.0 mA the optic nerve response to the test light was greatly enhanced by "inside cathode" and greatly diminished by "inside anode." This is shown in Fig. 3. With the whole nerve and relatively low sensitivity the effects at "on" and "off" completely dominate the picture of the optic nerve response of the light-adapted eye. The range in mA within which

these effects were obtained varied from eye to eye. Weak currents had little effect and stronger ones introduced various complications. On increasing current strength above the limits mentioned the typical effect was a diminution of the responses to both "inside cathode" and "anode," relatively greater for the latter. With "inside anode" and gradually increasing current strength the impulses could be almost completely blocked before the response to "inside cathode" had diminished by more than 50 per cent.

Differences in the relative effect of the polarizing current on the outburst of impulses at onset and removal of the stimulating light were fairly regularly seen. If, before polarization, the off-effects were large they increased or diminished in the same proportion as the on-outburst during the passage of the galvanic current. But if, to begin with, the off-effects were small, then they were more sensitive to the effect of polarization than the initial reaction to light. "Inside cathode" often made the off-effects relatively larger, and "inside anode" relatively smaller than the reactions to the onset of illumination. With very strong polarizing currents the off-effects were the last to disappear with "inside cathode" and the first to go with "inside anode."

2. *Nature of anomalous results.* Mixed atypical effects as well as reversal of the normal findings described above were sometimes obtained. But it proved possible to locate the source of error. The decisive factor turned out to be the amount of fluid or vitreous body at the inside electrode. Localization of this electrode to the retina immediately lead to unpredictable complex effects. These sometimes arose spontaneously when the cotton wick inside the bulb had drained away its content of fluid. In both cases normal results were immediately restored by the simple expedient of filling the bulb with Ringer solution.

From the point of view of the distribution of current between the poles of the galvanic source its concentration to a differential electrode touching the retina would seem to be a very complex affair. Our intention of placing the retina as a whole between the poles of a current distributed as uniformly as possible would seem to be realized only by the experiments in which the bulb was filled with fluid or the inside electrode was touching the lens floating in the middle of the opened eye.

3. *Effect of polarization on the retinal electrical response.* In order to find out whether polarization affected retina or nerve it was necessary to record the retinal electrical response itself. The leads to the amplifier were connected to the electrodes used for polarization and the compensatory device described in the section of methods was put in. Alternatively, in a few experiments, the input leads of the amplifier were blocked with condensers. Both methods gave identical results. "Inside cathode" was found to increase the retinal electrical response and "inside anode" to diminish it. In all essentials these facts were a replica of those obtained with the nerve. This is shown in Fig. 4. Hence polarization seems to affect the retina directly. The results obtained with the nerve must be due to changes in the retina.

The first effect of the polarizing current naturally was a drift of base line of the recording instrument. But as the effects of "inside cathode" and "inside anode" persisted for several minutes with exposure at intervals of 30 sec. it was possible to study them after compensation of this drift.

4. *Effect of polarization on the isolated negative component P III of the retinal response.* It is known (Granit and Therman, 1937; Therman, 1938) that a drop of KCl-solution applied on to the retina quickly removes the positive component P II of the electrical response to light and leaves a pure negative deflexion, which has the latency of the initial *a*-wave and returns to the base line in a way imitating the properties of the off-effect of the

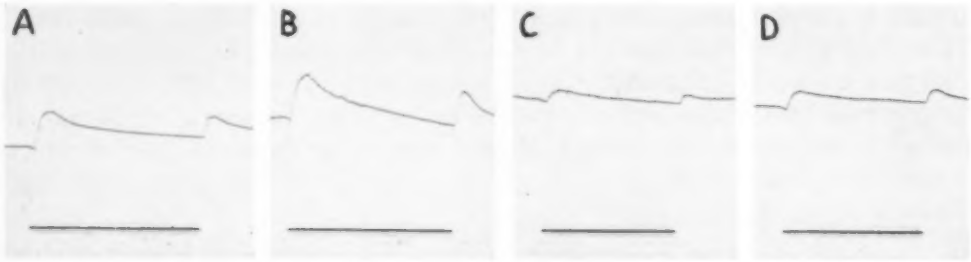

FIG. 4. Effect of polarizing on the retinal electrical response. A, normal electroretinogram; B, polarization (0.4 mA) with "inside cathode"; C, polarization (0.4) with "inside anode"; D, control. Note that the retinal electrical response has diminished from A to D. The characteristic effects of polarizing are nevertheless easily distinguished.

complete retinal action potential. This deflexion is identical with the negative component P III (Granit and Riddell, 1934). Before any change can be seen in the retinal action potential after potassium, the discharge in the optic nerve is blocked by the poison.

The eye gradually dies under the influence of a dose of KCl capable of removing selectively the positive component P II but the gradual disappearance of the negative P III is slow enough to enable a sufficient number of "anodes" and "cathodes" with intervening "controls" to be superimposed upon the slow decline of the electrical response to light. Figure 5 shows that the negative component P III reacts to polarization in precisely the same manner as the discharge in the nerve and the complete electrical response of positive sign. "Inside cathode" enhances, and "inside anode" depresses the negative component P III.

5. *Conclusion.* Let us consider what these facts mean in terms of the components of the retinal action potential. At the onset of illumination P II and P III are trying to deflect the galvanometer in opposite directions. If the two components were of equal potential and equally enhanced or diminished by polarization there would be complete cancellation of these effects at "on," whereas at "off" they would add. Thus, in general, "inside cathode" should favour the off-effects, and if, as we have seen, the *b*-wave also is larger it can only mean that the positive component P II has been

increased by "inside cathode" more than the negative component P III.

The increased retinal off-effect with "inside cathode" cannot be a simple interference phenomenon, due to P III returning from a lower level and without physiological significance as it is associated with an equivalent increase of the total discharge in the optic nerve.

6. *Antidromic impulses.* Already in 1932 one of us (R.G.) together with Dr. J. C. Eccles tried firing antidromically into the optic nerve of the decerebrate cat without being able to demonstrate any effect on the electro-

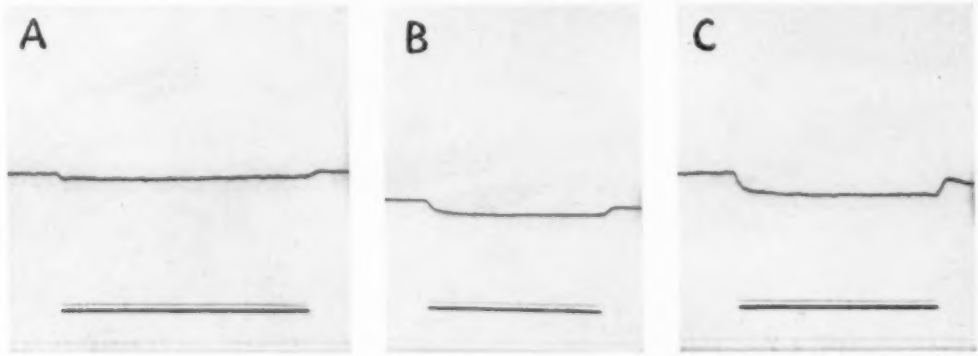

FIG. 5. Effect of polarizing on a retinal electrical response made negative (P III) by a drop of KCl into the eye. A, "inside anode" (0.4 mA); B, polarizing current removed; C, polarization with "inside cathode."

retinogram. These experiments were now repeated with the more accessible nerve of the frog's eye. The stimuli came from a neon stimulator, the cathode of which was lying towards the retinal end of the nerve. Widely different rates of stimulation were employed and the eye was illuminated for a few seconds at regular intervals leaving, as one would expect, the antidromic impulses a chance of being timed in every possible way relative to the onset of the retinal action potential. No effect whatsoever, excluding the rapid shock artefacts, was noticed. It is therefore hardly conceivable that the electroretinogram recorded with standard leads is due to slow potentials in the ganglion cells themselves. Hence it is also excluded that changes in the retinal response due to polarization are determined by effects of the polarizing current on this layer of cells.

7. *Relative latent periods of processes in retina and nerve.* It is difficult to argue in favour of any particular theory of the nature of the retinal electrical response from observations on the latent period in retina and nerve. Nevertheless some facts may be definitely established by this method. For measurements of latencies we used the double cathode ray and two balanced condenser-coupled amplifiers.

It was easy to confirm earlier observations by Adrian and Matthews (1927) and Granit and Therman (1935) to the effect that the negative component P III always precedes the discharge in the nerve. But it is impossible

to know what the positive P II does as its starting point merely shows the moment at which P III is compensated. This regularly occurs later than the first impulses are seen in the nerve. Despite this P II may really precede the nervous discharge.

But at "off" P II rises unimpeded by the simultaneous development of the negative P III. There, however, occurrence of positivity in the retina

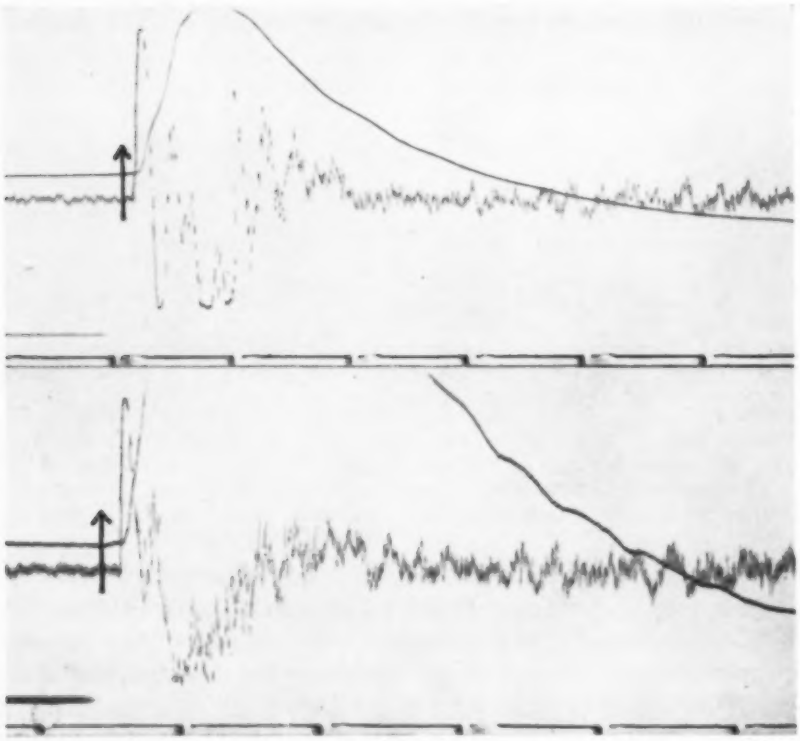

FIG. 6. Off-effects in retina and nerve. Simultaneous records taken with a pair of balanced condenser-coupled amplifiers from retina (upper cathode ray) and nerve (lower cathode ray). Lower record taken with very high amplification of retinal response. Note that retinal response begins with a small slowly rising phase, marked by arrow, before anything happens in the nerve. This phase is hardly visible when lower sensitivity is used, as in upper curve.

before the off-discharge appears in the nerve may indicate return of P III to the base line. As shown by Fig. 6 the appearance of the off-impulses in the nerve is regularly preceded by a small rise of positivity in the retina, but this is visible only at high sensitivity. The steep rising part of the retinal off-effect always follows a couple of milliseconds *after* the nervous discharge had begun. Selective activation of the negative component P III by a flash on top of the off-effect leads to inhibition of the impulses in the optic nerve, followed by a new retinal *b*-wave accompanied by a fresh discharge in the nerve. The inhibition, as is now well known (Granit and Therman, 1935;

Hartline, 1938), is associated with the characteristic initial *a*-wave of the retinal response. This wave belongs to the negative component P III (Granit and Riddell, 1934). This is shown in Fig. 7. The impulses stop more abruptly than the *a*-wave swings down in the retinal record. In the best records a comparison of the time relation of the two events shows that the *a*-wave in the retina just precedes the onset of inhibition in the nerve. The negativity therefore bears the same relation to inhibition as the small initial positivity to excitation.

Discussion

The two components of the vertebrate retinal response recorded in the standard leads must be localized to the region between the synapses of the ganglion cells and the receptors. The experiments with antidromic impulses would seem to exclude the ganglion cells themselves. It is difficult to imagine both components to be localized to the receptors, and equally difficult to exclude the receptors altogether. Then the retina of *Limulus polyphemus* with only one layer of cells gives one component alone, which with regard to its electrical sign corresponds to the P II of the vertebrate retina (Hartline, 1928; Hartline and Graham, 1932). From the phylogenetic point of view the rods and cones are primitive neurosensory cells (Kappers, Huber and Crosby, 1936), together with the olfactory cells the only ones of this kind carried through the whole progress of evolution. It would therefore seem necessary to identify the retinal response of *Limulus* with one component of the vertebrate electroretinogram. It is possible that the small initial rise of positivity in the retina preceding the off-discharge in the nerve is part of the component P II which is then occluded by the fast return of the negative P III towards the base line (cf. Granit and Therman, 1937).

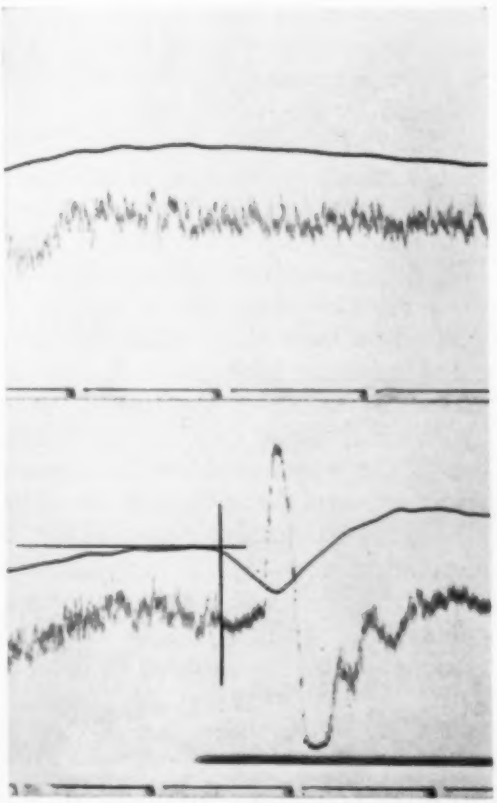

Fig. 7. Re-illumination during off-effect. Simultaneous records from retina (upper cathode ray) and nerve (lower cathode ray). Upper curve, off-effect control; lower curve, re-illumination during off-effect showing negative *a*-wave of P III in retina and cessation of discharge in nerve.

Recent work by Crescitelli and

Jahn (Jahn and Crescitelli, 1939; Crescitelli and Jahn, 1939) shows that the likewise complex retinae of the grasshopper (*Melanoplus differentialis*) and the moth (*Samia cecropia*) are characterized by retinograms in which the characteristic waves of the vertebrate response, the *a*-, *b*-, *c*-, and *d*-waves, can be identified. However, in the vertebrate retina the corresponding waves are of opposite sign, which probably only is due to the inversion of the latter. A component analysis of these insect retinograms is still lacking. Despite this it is tempting to suggest that one component of the vertebrate retina belongs to the receptorial layer or its synapses, the other one to the synaptic junctions of ganglion cells, amacrines or bipolars, perhaps to all of them. The main fact against placing any component in the receptors has been the evidence for synaptic interaction, demonstrable with the vertebrate retinal electrical response, but the assumption of electrotonic spread offers means of solving this dilemma. It is quite possible that the spread of excitation from the receptors onwards takes place by way of electrotonus rather than by impulses. Our results with polarization show it to be a reasonable proposition to assume that retinal excitability is influenced by electrotonic states.

Somewhat surprising is that "inside cathode," which means that the receptors are lying towards the anode, should cause a change in the direction of increased sensitivity. It reminds one of observations by Erlanger and Blair (1936), according to which anelectrotonus in peripheral nerve supplies a condition which makes it respond repetitively at a temporary cathode. The effects we are dealing with probably depend on ions being deposited on the cells or synapses when the current passes through the intercellular spaces. It is reasonable to assume that the active structures themselves are far less permeable to the galvanic current.

We had hoped that the experiments on polarization would help to solve one of the major problems in retinal electrophysiology: are P II and P III truly opposite potentials or are they merely of opposite sign because the structures generating potential are of opposite orientation relative to the electrodes? Our results make it difficult to understand how the sources generating P II and P III could turn different poles towards the polarizing current and yet be similarly influenced by the latter. It is at least far more reasonable to assume them to be of opposite sign.

Summary

The electroretinogram and the impulses from the optic nerve have been recorded during the passage of a galvanic current across the retina.

"Inside cathode" greatly enhances the discharge in the *optic nerve* and "inside anode" depresses it.

The complex *retinal* electrical response is also enhanced by "inside cathode" and depressed by "inside anode."

After removal of the positive component P II of the electroretinogram

the remaining negative P III reacts to the polarizing current just as the whole response.

Antidromic impulses through the optic nerve have no effect whatsoever on the electroretinogram.

Simultaneous records of the retinal and optic nerve responses illustrate the practically negligible nerve-retinal interval at cessation of illumination.

The off-discharge through the nerve is *preceded* by a small initial retinal positivity passing on into the large retinal off-effect the main part of which follows a few milliseconds *after* the discharge in the nerve.

A brief discussion of the localization of the components of the retinal electrical response concludes the paper.

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THE EFFECT OF ESERINE ON SPINAL REFLEXES IN THE DOG

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CURRENT interest in the action of eserine on the central nervous system derives largely from the acetycholine hypothesis of synaptic transmission. In terms of this hypothesis, eserine, by virtue of its anticholinesterase activity, would be expected to have some effect, probably excitatory, on somatic reflexes.

Reports concerning the central action of eserine have been contradictory, some workers having summarized the action as depression (Rothberger, 1901; Dixon and Ransom, 1924), and others as excitation (Langley and Kato, 1915; Schweitzer and Wright, 1937). The last named authors based their conclusion that eserine is a general excitant for the central nervous system on studies of the effect of the intravenous drug on the knee-jerk of chloralosed cats. In their experiments, however, they observed pure augmentation of the reflex only with the smallest doses, the larger doses producing mixed effects in which depression of the knee-jerk was a prominent feature.

In a preliminary study of the knee-jerk of the dog under barbital anesthesia, the most consistent effect of eserine was a depression of the reflex. We felt justified, therefore, in extending the study to include observations on a more stable reflex, that of the tibialis anticus. In the course of these studies, it was also possible to make some observations on the crossed extension reflex. Although our data are not definitive as regards the central action of eserine, they present the observation that even in the spinal state, flexor and extensor reflexes may be oppositely affected by a drug. It is largely for this reason, and as a criticism of the knee-jerk as a type somatic reflex, that these data are presented.

METHODS

Dogs were anesthetized with either sodium barbital (200–250 mg/kg) or chloralose (80 mg/kg). The knee-jerk was elicited with the solenoid hammer described by Johnson (1927) at intervals of 2, 4, 8, or 16 sec. in the different groups of experiments. At similar intervals the reflex response of the tibialis anticus was elicited by means of an A.C. stimulator of the type described by Bayliss and Eggleton (1935) applied to the posterior tibial nerve through shielded silver wire electrodes. The intensity of the stimulus was adjusted by means of suitable resistances. A rotating key was arranged to close the circuit for hammer or A.C. stimulator for a brief period (0.233 sec.; 14 cycles of the 60 cycle A.C.) at the desired intervals.

Both reflexes were recorded with partially isometric lever systems. In one group of experiments the two reflexes were recorded simultaneously in the same animal, in which case they were elicited in opposite legs, the rotating key being set so as to close the circuits alternately at intervals of 8 sec. In every experiment of this type one or the other circuit was held open from time to time during the observations to make sure that neither reflex

was influencing the other. In no case was there evidence of such influence, and the results of these experiments are identical with those in which either reflex was studied alone.

Eserine (physostigmine salicylate) was applied locally to the spinal cord by perfusing the lumbar subarachnoid space with Ringer's solution containing the drug in concentrations ranging from 0.025 to 0.2 per cent. The solutions were kept within 1–2 degrees of body temperature by means of a constant temperature bath. In these experiments the spinal dura was exposed by laminectomy and two No. 20 gauge spinal needles were inserted into the subarachnoid space, one at the 11th thoracic segment (inflow) and the other at the 1st sacral segment (outflow). In order to avoid the effects of pressure changes on the spinal cord, a continuous perfusion of Ringer's solution was started at the beginning of an experiment from a reservoir kept under constant pressure from a compressed air line with the aid of a mercury valve. A second reservoir at the same temperature and pressure, but containing eserine in Ringer's solution, was connected to the inflow needle through a two-way valve, so that instantaneous change of the perfusion fluid was possible. The dead space between the valve and the spinal subarachnoid space was 10 cc., which accounts for at least part of the observed latency of the response to subarachnoid perfusion of eserine. Perfusion pressure was adjusted to maintain a perfusion rate of 2–4 cc. per min., and a record of carotid blood pressure was taken to ensure that perfusion pressure was kept well below systemic blood pressure. In confirmation of Luckhardt and Montgomery (1929) it was found that pressure on the spinal cord produced no changes in the reflexes until this pressure attained the level of systemic blood pressure. It was attempted, by proper fixation of the needles, to keep the perfusion fluid from coming into contact with extrathecal tissues, but we cannot be sure that this was always completely successful.

In another group of experiments, the effects of intravenous eserine were studied. The drug was injected in a constant volume of Ringer's solution (5 cc.) in doses of 0.01–2.0 mg/kg.

Results

The significant observations are summarized in Table 1. As shown by

Table 1. Intravenous eserine

Reflex Observed	Number of Dogs	Number of Observations	Effect on Reflex	
			Augmentation	Depression
Knee Jerk				
(Barbital)	9	33	3	30
(Chloralose)	7	27	12	15
Tibialis Anticus				
(Barbital)	16	59	56	3
(Chloralose)	6	34	32	2
Intraspinal eserine				
Knee Jerk (Barbital or Chloralose)	8	16	2*	14
Tibialis Anticus (Barbital or Chloralose)	7	11	11	0

* Both these observations were made in one dog, non-spinal, under sodium barbital anesthesia. In this animal, the perfusion of eserine was followed by augmentation, depression, or no change of the reflex, with no apparent relationship between the concentration used and the effect produced.

this table, the usual effect of intravenous eserine on the knee-jerk of barbitalized dogs was depression. Successive injections of the drug were followed by further depression of the knee-jerk (Fig. 1). In no case were we able to demonstrate a reversal of the effect by increasing the dosage of eserine, beginning with doses so small as to be ineffective. Under chloralose anesthesia, however, the knee-jerk was augmented nearly as often as it was depressed. Since most of these observations were made on spinal animals (cord transected at T10), the effect of the anesthetic on the response to eserine must have been exerted on the spinal cord itself.

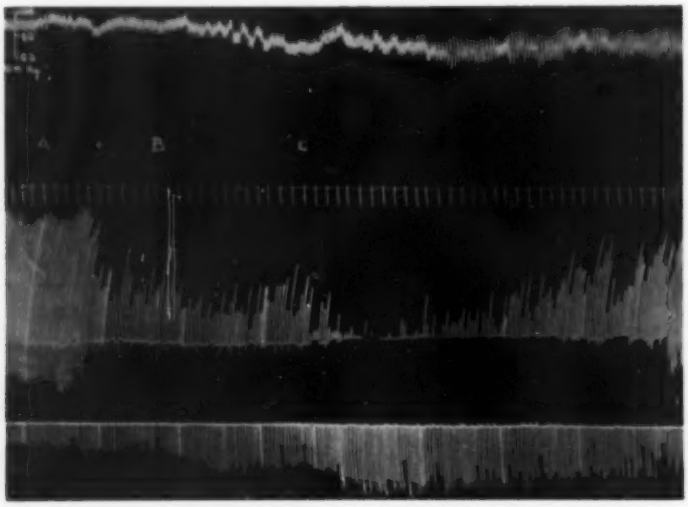

FIG. 1. INTRAVENOUS ESERINE. Dog, 12.3 kg. Sodium barbital, 200 mg/kg. Spinal cord sectioned at T10. Records from above downward are: 1) Carotid blood pressure. 2) Signal. 3) Time, in min. 4) Knee-jerk of left leg. 5) Tibialis anticus reflex of right leg, submaximal stimulus to posterior tibial nerve. At A, B, and C, eserine, 0.5 mg in 5 cc. Ringer's solution injected into jugular vein.

In both chloralosed and barbitalized dogs, intraspinal eserine depressed the knee-jerk in almost all cases (Table 1). In our short series no difference in the effect was apparent with the two anesthetics when eserine was administered by this route. The intraspinal application of eserine affected the knee-jerk, in most cases, before there was any effect on blood pressure or heart rate (Fig. 2), and in some cases, in the absence of such circulatory changes. The effect on the knee-jerk, therefore, is probably unrelated, in these experiments, to the systemic effects of eserine on the circulation. This is probably true also for intravenous eserine, since it was observed, in confirmation of Schweitzer and Wright (1937), that atropinization of the animal with reversal of the blood pressure response, did not affect the response of the knee-jerk.

In contrast with the variable, but preponderantly depressant, effect of eserine on the knee-jerk, the tibialis anticus reflex was consistently aug-

mented (Table 1; Fig. 1 and 2). This effect was observed equally well under barbital and under chloralose anesthesia, in the spinal and in the intact animal (anesthetized), and with both intravenous and intraspinal eserine. Complete atropinization did not abolish this augmentory effect of eserine. Large doses of eserine intravenously (1 mg/kg or more) sometimes caused a sustained increase in tone of the tibialis anticus which was accompanied by a diminished amplitude of the reflex response. In some of these cases, when tone returned to the normal level, the reflex showed some augmentation above the control level.

In some of the acute spnial preparations in which the knee-jerk and the tibialis anticus reflex of opposite legs were alternately elicited, the crossed

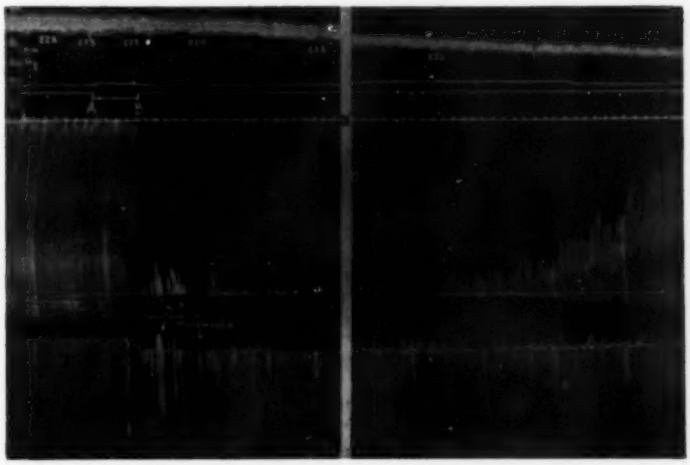

FIG. 2. INTRASPINAL ESERINE. Dog, 22.2 kg. Sodium barbital, 200 mg/kg. Spinal cord sectioned at T10. Records from above downward are: 1) Carotid blood pressure. Figures along this tracing are heart rates. 2) Perfusion pressure, 3) Signal. 4) Time, in min. 5) Knee-jerk, left leg. 6) Tibialis anticus reflex, right leg, submaximal stimulus. Between A and B, eserine, 0.1 per cent in Ringer's perfused through spinal subarachnoid space. Perfusion rate: 3.5 cc. per min.

extension reflex appeared in sufficient magnitude for observation. In these cases, the crossed reflex was augmented along with the flexion response, whereas the knee-jerk in that extremity was depressed. In some preparations, the crossed reflex appeared only after eserine was administered, and disappeared again as the other reflexes showed recovery from the effects of the drug.

A number of observations have been made on the effect of eserine on the response of the tibialis anticus and the quadriceps to stimulation of their motor nerves (A.C. stimulator). Simultaneous recording of the reflex response of the muscle of one leg and the response of that muscle in the opposite leg to stimulation of its motor nerve have shown effects on the reflex much earlier, or at lower dosage levels, than on the nerve-muscle response (Fig. 3). Even on intravenous administration, only the larger doses of

eserine had a demonstrable augmentory effect on the nerve muscle response. Our observations thus support the conclusion of Schweitzer and Wright (1937) that the effects described cannot be due to an action on the efferent limb of the reflex arc.

After the perfusion of eserine, the dogs often manifested the spontaneous muscular twitches reported by Langley and Kato (1915) to be of central origin. Such spontaneous activity was always confined to myotomes innervated from the perfused cord segments. This is taken as evidence that the action of eserine in these experiments is central rather than reflexly through

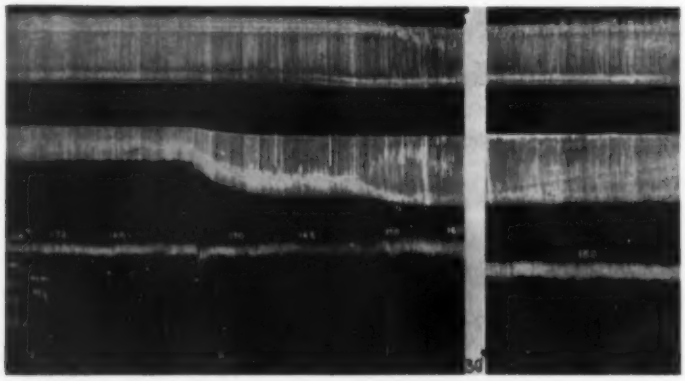

FIG. 3. INTRASPINAL ESERINE. Dog, 17.3 kg. Chloralose, 80 mg/kg. Spinal cord sectioned at T10. Records from above downward are: 1) Response of left tibialis anticus to submaximal stimulation of its motor nerve. 2) Response of right tibialis anticus to maximal stimulation of ipselateral posterior tibial nerve. 3) Carotid blood pressure. Figures along this tracing represent heart rates. 4) Perfusion pressure. 5) Signal. 6) Time, in min.

Between A and B, eserine, 0.1 per cent in Ringer's perfused through spinal subarachnoid space. Perfusion rate: 2.0 cc. per minute.

absorption and peripheral action, for in the latter case, segmental localization of the effect would not be observed.

DISCUSSION

The present data exclude the possibility that eserine acts on the efferent limb of the reflex arcs studied. It is considerably more difficult to dismiss the possibility that the drug, by virtue of its widespread visceral action, influences the cord reflexly. The fact that eserine in our study has identical effects on somatic reflexes in the spinal and in the intact animal argues against such a mechanism, since the spinal animal has lost most of its viscerosomatic reflexes. With sufficiently delicate indices to eserine absorption and systemic action, our data on eserine administered intrathecally would be conclusive evidence against this mode of action. Since our data on heart rate and blood pressure are admittedly crude indices, the possibility of absorption, with reflex effects on the cord, cannot be definitely ruled out. Persistence of the action of eserine on the reflexes in the face of full atropinization argues against this possibility. Furthermore, the strictly segmental

distribution of spontaneous muscular twitches following intraspinal eserine points to a localized central, rather than a disseminated peripheral, site of action.

It is tentatively concluded therefore, that the site of action of eserine in these experiments is the spinal centers. If this conclusion be justified, we are led to the important conclusion that this drug selectively augments some, and depresses other, spinal centers. Such selective action through afferent channels, or through effects directly on the higher levels of the neuraxis is not unusual. But we have not been able to find an account of any such action on the spinal cord itself.

The term "antagonistic reflexes" implies that any change in the one reflex will involve a reciprocal change in the other. According to the concept of the Oxford School (Creed *et al.*, 1932) such reciprocity as exists between the knee-jerk and the tibialis anticus reflex is a function of the motoneurone pools of the extensor and flexor muscles. Our data, however, cannot be interpreted entirely on such a basis, for one extensor response, the crossed extension reflex, is augmented coincidentally with the augmentation of the flexion response, whereas the other extensor reflex, the knee-jerk, is depressed. Since both these extensor reflexes are responses of the quadriceps muscle, they are presumably mediated by the same motoneurones. Unless we make the unlikely assumption that the quadriceps motoneurones are fractionated by the two reflexes so that each reflex is mediated by a special group, and that each of these groups is affected differently by eserine, it appears evident that the changes induced in the spinal cord by eserine could not have been confined to the motoneurones.

The conclusion seems inescapable that eserine acts somewhere upstream in the reflex arc, probably on internuncial pathways. On this basis, the depression of the knee-jerk would be regarded as the expression of some sort of inhibitory process taking place in the internuncial pool. Studies on cord potentials (Hughes and Gasser, 1934; Hughes, McCouch and Stewart, 1937) have presented excellent evidence that such inhibition may take place.

Although our knowledge as yet is far too incomplete for full interpretation of the observations, it is permissable to believe that the augmentation of the flexion and the crossed extension reflexes is related to the depression of the knee-jerk. Rather than postulate a selective difference of action of eserine on separate internuncial pools, a reasonable assumption might follow somewhat the lines of Gasser's theory of reciprocal inhibition (Gasser, 1937). According to this theory antagonistic reflexes share some link in the internuncial pathway, so that only one of them may use the common link at any given time. In the terms of this theory eserine might be regarded as having captured the internuncial link for the flexion reflex (and for crossed extension, which uses, in part, the same pathway), thus producing augmentation of these reflexes, and inhibition of the knee-jerk.

Regardless of final interpretation, these observations serve to indicate the inadvisability of characterizing the central action of a drug as excitatory

or depressant from evidence obtained through the study of single reflexes. It is obvious that eserine must be characterized as both excitant and depressant, depending on the reflex chosen. One other point worthy of mention is the discrepancy between the effects observed by us on the knee-jerk of the dog, and those observed by Schweitzer and Wright on the same reflex in the cat. We have no explanation to offer other than the possibility of species differences.

Summary and Conclusions

1) Studies have been made of the effect of eserine on the knee-jerk, tibialis anticus reflex, and the crossed quadriceps reflex in chloralosed and in barbitalized dogs.

2) Eserine, administered by perfusion through the lumbar subarachnoid space in the intact or spinal animal (cord transected at T10), depresses the knee-jerk and augments the flexion reflex in almost all cases. The crossed extension reflex is augmented in the extremity in which the knee-jerk is depressed.

3) Eserine given intravenously is predominantly depressant to the knee-jerk, and augmentory to the flexion reflex and the crossed extension reflex.

4) The possible locus of action and the significance of the results are discussed.

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* EEG is used for electroencephalogram; CNS for central nervous system.

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